A STUDY OF AUTUMN OLIVE (*ELAEAGNUS UMBELLATA)* PHENOLOGY AND ASSOCIATED PHYSIOLOGICAL TRAITS THAT MAY FACILITATE ITS INVASION OF THE UNDERSTORY OF A SOUTHERN APPALACHIAN FOREST

A Thesis
by
EMILY CAROLINE RIFFE

Submitted to the Graduate School
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

August 2018
Department of Biology

A STUDY OF AUTUMN OLIVE (*ELAEAGNUS UMBELLATA)* PHENOLOGY AND ASSOCIATED PHYSIOLOGICAL TRAITS THAT MAY FACILITATE ITS INVASION OF THE UNDERSTORY OF A SOUTHERN APPALACHIAN FOREST

A Thesis
by
EMILY CAROLINE RIFFE
August 2018

APPROVED BY:

Howard S. Neufeld, Ph.D.
Chairperson, Thesis Committee

Julie C. Zinnert, Ph.D.
Member, Thesis Committee

Annkatrin Rose, Ph.D.
Member, Thesis Committee

Zack E. Murrell, Ph.D.
Chairperson, Department of Biology

Michael J. McKenzie, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Copyright by Emily Caroline Riffe 2018
All Rights Reserved

Abstract

A STUDY OF AUTUMN OLIVE (*ELAEAGNUS UMBELLATA)* PHENOLOGY AND ASSOCIATED PHYSIOLOGICAL TRAITS THAT MAY FACILITATE ITS INVASION OF THE UNDERSTORY OF A SOUTHERN APPALACHIAN FOREST

Emily Riffe
A.A., Rowan Cabarrus Community College
B.S., North Carolina State University

Chairperson: Dr. Howard S. Neufeld

Elaeagnus umbellata, better known as Autumn Olive (AO), is an exotic tree species that has invaded much of the eastern United States. It grows in a variety of habitats, but little is known about its ability to invade and persist in forest understories, especially at high elevations in the southern Appalachians. AO may take advantage of high light conditions before canopy leaf out and after canopy leaf fall by leafing out early and maintaining leaves late into the fall. This would allow AO access to high light in early spring and late fall, when it may gain most of its carbon. To address this hypothesis, I made phenological and gas exchange measurements throughout 2017. AO began leafing out in mid-February whereas native understory species didn't leaf out until early April, and canopy trees until mid-April. Peak leaf number for AO was reached in early June and survivorship followed a Type I curve through the season, with 6% of leaves still remaining by mid-November. I also

measured diurnal patterns of gas exchange at approximately monthly intervals using the Li-6800 gas exchange system. Peak carbon gain of 161 mmol CO_2 m^{-2} day^{-1} occurred in May while AO had an average carbon gain of only 11 mmol CO_2 m^{-2} day^{-1} during the summer months, when light levels were much lower. The carbon gain increased again during the fall with an October rate of 91 mmol CO_2 m^{-2} day^{-1}. As hypothesized, rates of carbon dioxide uptake by AO were higher in spring and fall when light levels were higher versus in summer when they were lower, suggesting that most of its annual carbon is gained during those periods when the overstory is leafless. The nearly two months of extended phenology gives AO a physiological advantage over native species, thereby contributing to its invasiveness. Autumn Olive is also a known nitrogen fixer; however, this process usually demands high light levels because it is so energy expensive for the plant, and it is unknown if AO fixed nitrogen in understory habitats. I completed indirect measures of fixation by measuring C:N, %N, and $\delta^{15}N$ of the leaves in comparison to native congeners. These measures were indicative that the plant is likely capable of fixing nitrogen while inhabiting the understory, however they are inconclusive. This project helped identify that the main method of understory invasion for autumn olive is the use of an extended leaf phenology.

Acknowledgments

Thank you first and foremost to Dr. Howard Neufeld for being my guiding light through this process. Thank you to my committee members Drs. Julie Zinnert and Annkatrin Rose for all their help in making this possible, and Dr. Jennifer Chandler for teaching me everything I know about hemispherical photos. Dr. Mike Madritch was of great assistance with my nitrogen analyses. I thank Mike Hughes and Dana Green for their technical assistance putting up a weather station at my field site. Andrew Jenkins was invaluable for being able to identify any leaf I brought to him. My fellow plant ecophysiologists in the Neufeld lab, thank you for sharing my passion and living through the struggle with me. Specifically, I want to thank Ivy Culver for being my field partner throughout all of 2017. I thank my family for giving me encouragement to continue my education, and most importantly, I want to thank Justin Ferrullo for dealing with the daily complaints, for helping me with field work, and for learning more about plants than he ever intended to. Funding for this research was provided by the Appalachian State University Office of Student Research, the ASU Graduate Student Association, the Department of Biology at ASU, the Southern Appalachian Botanical Society, and the North Carolina Invasive Plant Council.

Dedication

I dedicate this thesis to Dr. Sarah Marshburn for being my rock for the two years I was at Appalachian State University. I couldn't have written this without her constant encouragement. Sarah is by far the most genuine and honorable person I've ever met. She has been an inspiration to me and I'm grateful for having had the opportunity to work with her.

Table of Contents

Foreword

This work was completed in collaboration with a fellow master's student, Ivy Culver, to better understand the survival of invasive species in high elevation forest understories. The format and references follow that of the journal Biological Invasions.

Introduction

Invasive plants are an increasing environmental and economic issue in our society (Dornbos et al. 2016). In the United States, an invasive species is described as "*…a non-native organism whose introduction causes or is likely to cause economic or environmental harm, or harm to human, animal, or plant health.*" (Executive Order No. 13112, 1999; Executive Order No. 13751, 2016). However, this is a global issue and the definition varies depending on who you ask, which results in relatively minimal regulation of invasive plants (Reichard and White 2001). This makes understanding mechanisms of success for known invasive species critical as well as allowing the identification of a potential new invasive species.

Invasive plants cost the United States $138 billion dollars a year from loss of ecosystem goods, services, and processes (Pimentel et al. 2005; Dornbos et al. 2016). They also cause a variety of environmental issues by taking over habitats typically dominated by native plants. This leads to loss of habitat and food for animals, loss of biodiversity, and can be detrimental to specialist pollinators (Traveset and Richardson 2006; Pyšek et al. 2012).

The mechanisms which allow some exotic species to become invasive are only recently coming to light. There is still no comprehensive theory that can accurately predict which species will become invasive and which will not (Warren et al. 2018). However, there are some commonalities among invasive plant species that may allow them to gain an advantage over native species, and among these factors are: greater drought, cold or heat tolerances, more efficient dispersal methods, extended leaf phenologies, and the ability to fix nitrogen (Blossey and Notzold 1995; Kean

and Crawley 2002; Callaway and Ridenour 2004; van Kleunen et al. 2010; Warren et al. 2018). Nonetheless, not all species possess all attributes and some species with some of these attributes are non-invasive (Westoby et al. 2002; Orr et al. 2005; Brym et al. 2011).

There has also been research on the correlation between invasiveness and the region of origin of the species. Herberling et al. (2017) found that plants from East Asia (EAS) are frequently invasive when introduced into eastern North America (ENA). In fact, 29% of the invasives in ENA are from EAS. They also found that 56% of these EAS invaders are woody species. This could be due to similar climatic conditions at the latitude of their origin, or environments that are becoming more similar due to climate change (Warren et al. 2018). Another research consideration is to identify environments with attributes common for invasive success, so that they can be monitored for the future (Ibáñez et al. 2009). Researching the physiology of a species allows us to consider both aspects simultaneously (factors that put a species at risk for invasion and areas at risk for invasion). Recognizing the physiological differences and range of responses in a species allows for identification of optimal environmental factors for the spread of the species, and the detection of areas that have the identified factors and could be at risk from invasion.

Autumn olive (*Elaeagnus umbellata*; AO) is an exotic species native to EAS, specifically Pakistan, China, Afghanistan, India, Korea, and Japan (Edgin and John 2001; Ahmad et al. 2006). This species was introduced to the United States in 1830 as an ornamental shrub and was planted for erosion control (Orr et al. 2005; Dornbos et al. 2016). This is a common form of introduction and it has been found

that 82% of woody invasives were introduced for horticulture purposes and 3% for erosion control (Reichard 1997; Reichard and White 2001). In its native habitat, 10 different ecotypes have been identified for commercial benefits as it is used for fuel, fencing, baskets, and food (Ahmad et al. 2006). AO has fleshy fruits that have been shown to contain nutritional and medicinal properties (Ahmad et al. 2006) and they are also readily dispersed by birds (Bonilla and Pringle 2015).

Fig. 1 Autumn olive branch from August 2017
(State Nature Preserve, ASU Campus. Photo by author.)

Since its introduction in 1830, AO has become one of the most noxious invasive woody exotics throughout much of the United States and Canada (Catling et al. 1997). It is capable of growing both in full sun and in shaded habitats (Yates et al. 2004; Dornbos et al. 2016) and has the ability to rapidly produce very dense thickets, even in the understory, as documented in Michigan where it increased its density by six times and its spread by 26% in just two years (Dornbos et al. 2016). Autumn olive is a drought and salt tolerant species and tends to prefer coarse, well drained soils (Ahmad 2006; Naumann et al. 2010). However, it is capable of growing in a variety of soil textures with pH ranging from 4 to 8 (Ahmad 2006).

The reasons for AO's invasiveness are still poorly understood. One common hypothesis for invasiveness is the "*release from natural enemies*" hypothesis where an introduced species is no longer constrained by a major predator/herbivore in its new habitat (Keane and Crawley 2002). Brym et al. (2011) did observe anecdotally that leaves of AO rarely suffered herbivory. However, it is unlikely that this feature alone would suffice to constrain the spread of AO in the U.S. Another is allelopathy, but a study on this did not show definitive results (Orr et al. 2005). Thus, other aspects of the ecology of AO may play more important roles in facilitating its spread and persistence.

Most of the research on AO in the United States has concentrated on its invasiveness in open field habitats (Zinnert et al. 2013), even though it is capable of invading and dominating the poorly lit environment of forest understory habitats. As a result, little is known about the mechanisms by which it is able to invade and persist in the relatively light-limited understory of eastern forests (Dornbos et al. 2016). In the State Nature Preserve on the campus of Appalachian State University in Boone, NC, this species has been rapidly increasing its presence in the understory. In less than 5 years, it has nearly quintupled its areal coverage (Howard Neufeld, personal observations) and it is this rapid spread that was the emphasis for conducting the research described in this thesis.

In Michigan, Brym et al. (2011) looked at a variety of leaf traits of AO inhabiting the understory in comparison to native understory species. Their results showed that the specific leaf area, leaf laminar area, and leaf nitrogen content of AO leaves matched those of native shade-intolerant species, rather than those for congeneric

native understory species. This led them to conclude that AO is not necessarily "*shade-adapted*" but is using other strategies to invade these shady areas (Brym et al. 2011), such as release from natural enemies, nitrogen fixation, more efficient dispersal and possible use of high light early in the growing season.

Fridley (2012) was among the first researchers to focus on extended phenologies as a key strategy for the competiveness of non-native invasive woody species, whereby invasives leaf out earlier in the spring and retain leaves for longer in the fall then native congeners (Chen and Matter 2017). Extended leaf phenology is becoming recognized as a pivotal adaptation for invading plants, especially in the Eastern United States (Fridley 2012; Smith 2013; Gallinat et al. 2015; Chen and Matter 2017), and has been observed in *E. umbellata* and three other *Elaeagnus* species in a large comparative study by Fridley (2012), conducted in upstate New York. Fridley found that it was more common for non-natives than natives to have extended leaf phenology in the fall than the spring. Using a physiological modeling exercise, he also postulated that the extended leaf phenology of exotics in the spring was not as significant as in the fall as compared to native species in terms of carbon gain by the plants. However, personal observations by the author have shown that AO leafs out very early in the southern Appalachian Mountains (mid-February), which suggests that in the southern portion of its range, its extended spring phenology could play a larger role than it does in the north.

Autumn olive is also a known nitrogen fixer due to its endosymbiotic relationship with the bacteria *Frankia* (Naumann et al. 2010). Nitrogen fixation can result in a higher leaf N content (Evans 1989), which would allow for higher

photosynthetic rates due to increased chlorophyll and RUBISCO contents. It also allows AO to persist in areas with poor, eroded soils (Ahmad et al. 2006) and may give it an advantage over native species in such conditions. This attribute has also led to AO being used as a nurse plant for some species, often black walnut (*Juglans nigra*), which has increased AO's spread in the US (Funk et al. 1979).

However, due to the high energy requirement for nitrogen fixation, this process is often limited in the shade (Dixon and Kahn 2004). Therefore, it is unclear if much or any nitrogen fixation occurs in AO when it grows in understory habitats compared to what it achieves in full-sun locations. It is possible that nitrogen fixation varies seasonally and may only be active when the canopy is leafless and light levels are higher. Byrm et al. (2011) did show that AO leaves collected in the understory had traits consistent with the occurrence of some nitrogen fixation, but it is not clear when this fixation might have occurred. Even if AO is able to fix nitrogen only seasonally this could still give it an advantage over native species.

Fig. 2 Understory thicket of autumn olive from May 2017
(State Nature Preserve, ASU Campus. Photo by Howard Neufeld.)

The objective of my research was to assess the physiological mechanisms that allow AO to persist and thrive in the shaded forest understory. The field site was located in a cove hardwood forest understory of the Appalachian State University

Nature Preserve in Boone, NC where AO has formed a dense thicket that is rapidly expanding in size.

For my thesis research, I tested three hypotheses:

1. The ability of AO to persist and thrive in this environment is largely due to its extended leaf phenology compared to co-occurring native species. This would allow it access to high light when other species are dormant, such as in early spring before the canopy leafs out, and in autumn, after the canopy has lost its leaves, giving AO a competitive advantage over native congeners.
2. AO takes advantage of this extended leaf phenology by gaining most of its annual carbon during these two high light periods (i.e. early spring/late fall) when the native species have yet to leaf out (Brym et al. 2011). This carbon gain produces a pool of carbohydrates that allows it to persist through the shaded months of the summer, when it cannot attain high levels of photosynthesis.
3. AO is able to fix nitrogen in the understory, which assists with its invasion into this shaded habitat.

Methods

Study Site

The study site was located in the Appalachian State University Nature Preserve (36.2130°, -81.6910°, 1053 m) which comprises 27 ha of protected land that is situated adjacent to the west side of the campus. It consists of successional forests that are dominated by a variety of native tree species, including: red maple (*Acer rubrum*), sugar maple (*A. saccharum*), black locust (*Robinia pseudoacacia*), tulip polar (*Liriodendron tulipifera*), hemlock (*Tsuga canadensis*), and hickory mockernut (*Carya tomentosa*). The shrub understory consists mostly of great laurel (*Rhododendron maximum)* and several invasive shrubs/trees: autumn olive (*Elaeagnus umbellata)*, multiflora rose (*Rosa multiflora*), and Japanese barberry (*Berberis thunbergii*). The invasive species, particularly the autumn olive (AO), can form very dense thickets which do not allow for much light to get through to the floor of the understory, which makes it difficult for native wildflowers to grow, such as: wild geranium (*Geranium maculatum*), violets (*Viola* spp.), showy orchis (*Galearis spectabilis*), and mayapple (*Podophyllum peltatum*), to name but a few.

Autumn olive exists primarily in a small stand in the northern portion of the preserve at an elevation of 1053 m, which is about 24 m below the highest point in the preserve. Most of the plants are located on the lower mid-slope on either side of the trail through this section, and this is where I marked plants for field measurements. In the beginning of 2017 I randomly chose 15 plants along the trail to use for all of my measurements and all data were collected throughout the 2017 growing year. On each of those 15 plants I randomly selected 5 branches to use for

phenology measurements. Also from these plants I randomly selected 5 to use for diurnal gas exchange measurements.

Weather data for the stand were obtained using a Vantage Pro II weather station (Davis 6152; Hayward, CA), mounted 2 m up on the trunk of a tree near the center of the AO stand. Data collected included light (W/m^2), temperature, humidity, wind speed, and direction.

Overstory Development

Overstory development was monitored using hemispherical photography to document changes in light penetrating to the understory. The hemispherical photographs were taken with a Canon EOS Rebel T2i camera (Canon, Inc., Tokyo, Japan) and a 180° fisheye lens (Sigma 4.5mm F2.8 EXDC; Sigma Corporation of America, Ronkonkoma, NY). The hemispherical photos were taken three times throughout the season: (1) before the canopy filled in (April), (2) with canopy fully leafed out (July), and (3) as the canopy began to lose its leaves for the fall (October). For these photos, I placed the camera ~1 m from the forest floor on a tripod, and oriented it toward magnetic North. I took one photo near each of the 15 AO plants that I was using for the phenology measurements. These photos allow for a visual comparison of the amount of direct radiation reaching the AO plants for each of the three phases of canopy development over the growing season. These photos were later analyzed using ImageJ (NIH; Washington, D.C.) to calculate percent open sky. I also took instantaneous measurements of photosynthetically active radiation (PAR) using a quantum sensor (Li-190r; Licor, Inc., Lincoln, NE)

connected to a Li-250 light meter, once a week, between 10 am and 2 pm weather permitting. This allowed me to obtain an average PAR for each of the 15 plants throughout the entire growing season from February to November 2017.

Phenological Methodologies

I took measurements of the phenology of the 15 AO plants mentioned above in the understory throughout the growing season of 2017. Occasionally, I had to replace marked branches due to weather related losses. Once the buds started to break in February I assessed the stage of leaf out weekly, characterizing the initial stages as either swollen, exposed, or flushed. A count of each type of bud was taken on each of the 75 branches. Once the majority of the leaves were fully developed (which occurred by April 18th), I switched to completing weekly leaf counts throughout the rest of the season. In the beginning of May there was a severe storm and two plants were knocked down by the wind, lowering my total plant count to 13. These weekly leaf counts allowed me to assess any leaf growth in the spring or summer as well as the rate of leaf loss so I could calculate survivorship throughout the season.

Leaf Pigments, Leaf Mass per Area, and Nitrogen

Chlorophyll content (chl) (µg cm^{-2}) and leaf mass per area (g cm^{-2}) were measured periodically throughout the growing season: (1) before the canopy closed (April 16th), (2) when the canopy was fully closed (July 11th), and (3) after canopy leaves had mostly fallen (October 20th).

I extracted chlorophyll from three leaf punches per plant (0.84 cm^2 total leaf area) from each of the 15 AO plants in 3 mL of DMF (*N,N*-Dimethylformamide) in the dark, in a refrigerator at 5^oC for a minimum of 24 hours. Absorbances were measured using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Chlorophyll concentrations were calculated according to equations in Porra (2002).

Five leaf punches (1.40 cm^2 total leaf area) from 10 of the AO plants were used to obtain the leaf mass per area (g cm^{-2}), after drying at 65^oC for 24 hrs in a Thelco precision model 17 drying oven (Thelco Technology, LLC, Chicago, IL) and weighed on a Sartorius Practum scale (Sartorius, Goettingen, Germany).

Leaf samples were sent off for analysis of stable nitrogen isotope ratios ($^{15}N/^{14}N$) to evaluate whether or not AO was capable of fixing nitrogen in the understory (Diaz-Barradas et al. 2015). These samples were sent to the Colorado Plateau Stable Isotope Laboratory in Flagstaff, AZ. If AO was fixing nitrogen, then its $\delta^{15}N$ values should be less negative and closer to zero than the congeneric species that are not nitrogen fixers (Delwiche et al. 1979; Unkovich 2013).

AO leaves were collected in March and in July to determine if there were any seasonal differences in isotope concentrations which might reflect variation in N-fixation rates. Leaves from several other woody species in the preserve were also collected for comparison. The co-occurring species chosen were far enough away (> 10 m) from the AO as to not be affected by possible fixation and incorporation into the soil and subsequent uptake by these species. The species chosen included: red maple, sugar maple, red oak (*Quercus rubra*), black locust, American ash (*Fraxinus*

americana), and hickory mockernut. These species were only collected in July because they had not yet leafed out in March. Before sending the leaves to the stable isotope laboratory they were freeze-dried in a LABCONCO Freeze Dryer (Labconco corporation, Kansas City, MO), placed in plastic tubes with 5 stainless steel BBs, ground using a Pacer industrial mixer (Pacer Industrial, Inver Grove Heights, MN), weighed between 4-6 mg, and placed in tin capsules for analysis.

Soil samples were also collected for total nitrogen content, with the idea being that if AO is fixing nitrogen, the soils beneath its canopy should be enriched in this element. These samples were collected in January of 2018. A total of 20 samples were collected, with 10 inside the stand of AO and 10 from > 10 m outside the stand, but at the same contour. These samples were air-dried at 65°C for one week, then ground and weighed using the same methods as for the isotope preparation. Samples were analyzed on a Flash EA1112 CN analyzer (Thermo Fisher Scientific, Waltham, MA).

Water Stress

During July and August, the two warmest months, I measured diurnal water potentials of 5 AO plants to determine their maximum seasonal water stress, using a Scholander pressure chamber (PMS Inc., Corvallis, OR). These measurements were taken on the same days that I completed diurnal measurements for those months. The measurements were begun starting at about 8:00 am EST and at about three hour intervals till 6:00 pm EST.

Gas Exchange Measurements: Diurnals

Gas exchange measurements were made using the Li-6800 portable gas exchange system equipped with the 6 cm^2 chamber with LED lighting. Diurnal patterns of photosynthesis (A) and stomatal conductance (g_s) were made at approximately 3 hour intervals during the day from 7 am to 7 pm, on three leaves on each of 5 AO plants, once a month (weather permitting) from April to October. The five plants used were randomly chosen at the beginning of spring 2017 and the same five plants were used throughout the season. Leaves were chosen randomly on each plant, while avoiding using the same leaf multiple times a day. Values for the three leaves were averaged to obtain a plant average at each measurement time and this value was used in all subsequent statistical analyses.

Cuvette parameters were set to match ambient light and ambient temperature and incremented throughout the day as these changed, while CO_2 was kept constant at 400 µmol mol^{-1}. However, if there was a change in ambient light of over 100 µmol m^{-2} s^{-1} (mostly due to shading), then I would adjust the light. The relative humidity was set as close as possible to match the ambient humidity, but adjusted to avoid excess moisture in the system, and ranged from 10% in October to 70% in June; summer months typically had higher humidity, except for July which was only at 15%.

To calculate the daily carbon assimilation rate for individual plants, I integrated the area under each diurnal curve, using linear extrapolation between points and geometry. When rates were negative, i.e., early in the morning and late in the afternoon, those integrated carbon values were subtracted from the other totals

where *A* was positive. These values were averaged for the five measured plants on each day.

Gas Exchange Measurements: Response Curves

Light, temperature, and vapor pressure deficit (VPD) curves were assessed three times throughout the season: (1) before the canopy closed (May), (2) when the canopy was fully closed (July), and (3) after canopy leaves had mostly fallen (October). For all curves, cuvette CO_2 was kept constant at 400 µmol mol^{-1}. Measurements were usually completed before 2 pm to avoid diurnal influences.

Light Response Curves

Light response curves were measured on 3-5 plants per period. The light levels, in the order used, were: ambient for that day, 2000, 1500, 1250, 1000, 750, 500, 300, 150, 50 and 0 (µmol m^{-2} s^{-1}). Temperature was set to reflect the ambient temperature of that day while VPD varied depending on the ambient humidity of that day, but ranged between 2.77 and 1.22 kPa.

A 3-parameter exponential rise to maximum equation was used to fit each light response curve using SigmaPlot Ver. 12.5 (Systat Software Inc., San Jose, CA):

$$[y = y_0 + a(1 - e^{-bx})]$$

From this, I extracted the dark respiration rate (at zero PAR), light compensation point (where $A_{net} = 0$), apparent quantum efficiency (slope derived from linear regression of first three points), A_{max} (average of four highest rates of A_{net}), and saturation light intensity (where A_{net} = 97% of A_{max}).

Temperature and VPD Response Curves

I also generated temperature and VPD response curves on three randomly selected plants. Temperatures that could be obtained varied depending on the weather that particular day. Low temperatures were higher in July than during May because of problems with condensation in the system. Regardless, I was able to achieve a 20°C range for each sampling day. For the May measurement that was between 10°C and 30°C, for July it was between 20°C and 40°C, and for October it was roughly between 15°C and 35°C. Measurements began at ambient temperature, before dropping down to the lowest temperature and then being increased in 5°C intervals. The light for these measurements was set at 750 µmol m^{-2} s^{-1} for May and 400 µmol m^{-2} s^{-1} for July and October. The light saturation point for this species was ~750 µmol m^{-2} s^{-1}, but I did not use this light level for the July and October measurements because it could have shocked the plants that had been receiving much lower light levels during the middle and end of the growing season. The humidity was kept around 10 mmol mol^{-1} to minimize condensation.

VPD response curves covered the range from 1 to 3 kPa. Measurements began at ~1.5 kPa before dropping to 1 kPa and then being raised in 0.5 intervals to the highest VPD possible. For the October measurement, the lowest VPD I was able to measure was 1.5 kPa. Light was kept constant at 1000 µmol m^{-2} s^{-1} and temperature was set to 25°C.

For both temperature and VPD I fit 2nd degree polynomial functions to the response curves using SigmaPlot Ver. 12.5:

$$[y = y_0 + ax + bx^2]$$

Statistical Analysis

Statistical analyses were completed and figures created using Sigmaplot 12.5. Calculation of means and standard errors were used to compare trends for the phenology data, weekly PAR, and diurnal measurements. I performed two-way ANOVAs using month and plant as the factors for chlorophyll content and leaf mass per area. A one-way ANOVA with time as the main factor was used to analyze seasonal changes in light curve parameters and leaf nitrogen content. A Mann-Whitney Rank Sum Test was used to analyze for the difference in soil nitrogen content inside vs outside the stand of AO. For all analyses $p \leq 0.05$ was used for significance.

Results

Overstory Development

Overstory development was measured using hemispherical photos (Fig. 3) and measurements of PAR over each sampled individual plant. The percent of open sky calculated from the hemispherical photos allows for a comparison of canopy cover in April, July, and October, covering those periods prior to leaf out, at peak coverage, and during leaf fall in autumn. There was a significant ($p < 0.001$) difference between all 3 months. Prior to leaf out in April, percent open sky average 69.1 ± 1.08 %, whereas in mid-summer in July, it was reduced to just 20.2 ± 1.34 %. Once leaves began falling in October, the percent open sky increased again to 35.7 ± 1.62%, which was not as much as in April, due to partial retention of leaves at this time of the year.

Fig. 3 Hemispherical photos of the canopy at the same location from months April, July, and October 2017

The weekly hand-held PAR measurements in the understory allowed comparison of canopy change effects throughout the growing season. Figure 4A shows changing PAR in the understory throughout the season. The highest PAR readings (1312 µmol m^{-2} s^{-1}) were found early in the spring before canopy leaf out, and then levels started to drop significantly beginning on April 8th, as the canopy began to leaf out. Large variations in light in any season result from clouds, which are extremely common throughout the day in this part of the country.

Maximum PAR was < 75 µmol m^{-2} s^{-1} from June 2nd through September 22nd, but the lowest PAR levels, which were found on July 7th, possibly indicate peak canopy leaf area at this time of year. PAR started to rise again around September 28th as the canopy began to lose leaves, and then rose sharply to 447 µmol m^{-2} s^{-1} on October 27th, by which time most canopy trees had lost their leaves.

Phenology

Autumn olive began bud break by February 14th and was 98% leafed out by April 12th (Fig. 4B). If we compare leaf out to PAR (Fig. 4B, 4A), it is apparent that AO was fully flushed by the time the overstory canopy had begun to leaf out, which means that from February 14th until April 8th (53 days) AO had leaves that experienced high light before the canopy began to shade them. Fully flushed AO plants experienced 16 days with PAR $\geq$ 600 µmol m^{-2} s^{-1} before the canopy started to fill in, and then another 10 days with PAR over 400 µmol m^{-2} s^{-1}. The only other plants that were leafing out in April were two other invasive congeners, barberry and multiflora rose.

Leaf Survivorship

All 13 of the plants that I measured for leaf phenology exhibited Type I leaf survivorship curves (Rauschert 2010; Fig. 4C), characterized by minimal loss of leaves in the early to mid-portion of the season, followed by a rapid decline at the end of the season. The primary reason for mid-summer leaf loss was storms. Leaf number peaked at the beginning of June and remained above 50% until October 20th and by the end of November, all of the leaves had senesced.

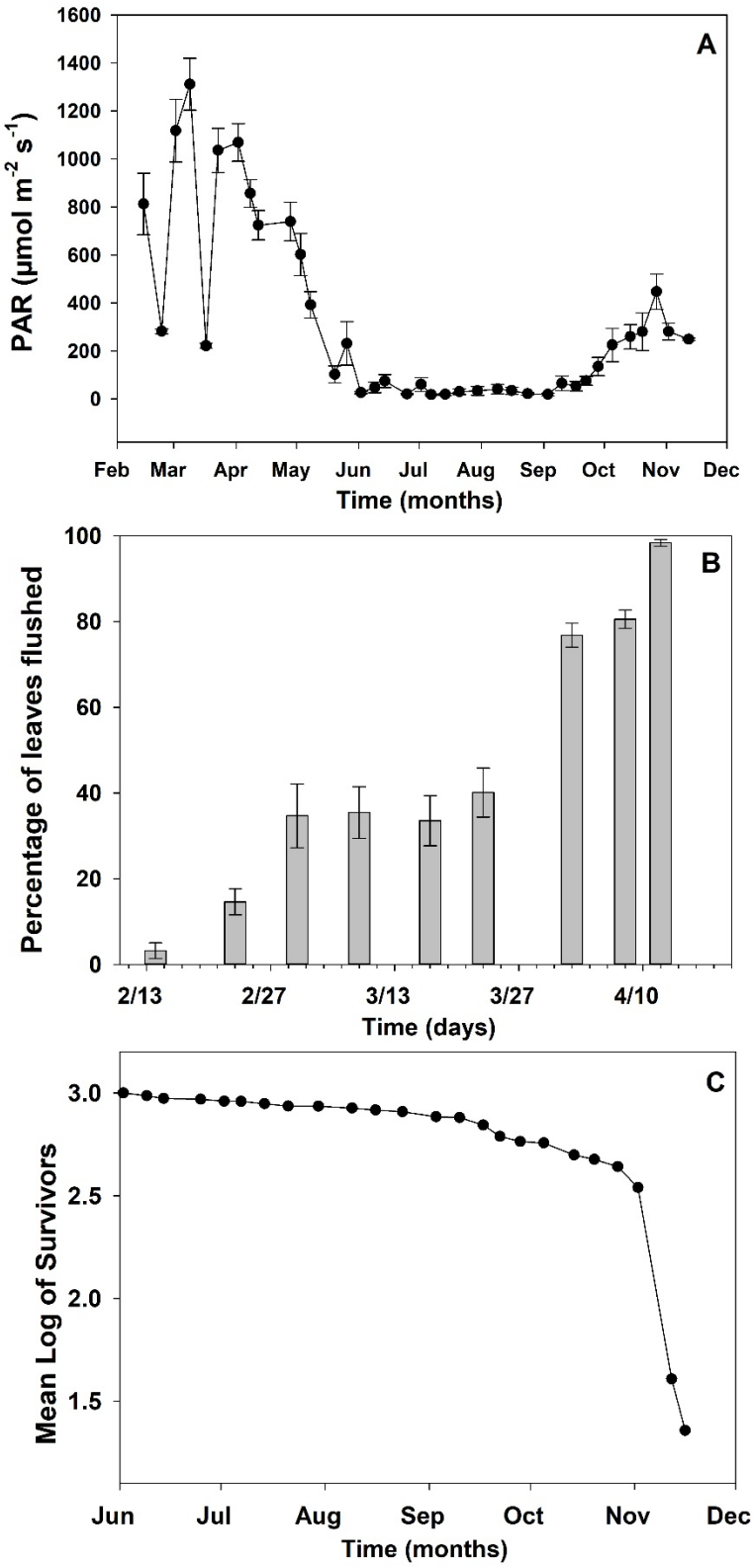

Fig. 4 Understory light levels (A), leaf out (B), and leaf survivorship graphs (C) for the 2017 growing season

Leaf Pigments, Leaf Mass per Area, and Nitrogen

There were no significant differences in the monthly means for chl *a*, chl *b*, or total chl concentrations in AO from April through October. Total chl ranged from 6.60 ± 0.20 µg cm^{-2} in April to 5.80 ± 0.66 µg cm^{-2} in October. The chl a:b ratio was significantly (p = 0.018) lower in July (3.77 ± 0.04 µg cm^{-2}) than in April (4.73 ± 0.08 µg cm^{-2}) but the October chl a:b ratio did not differ from either April or July (4.51 ± 0.32 µg cm^{-2}). Carotenoids also showed a seasonal trend and were significantly (p < 0.001) lower in July than either April or October (Fig. 5).

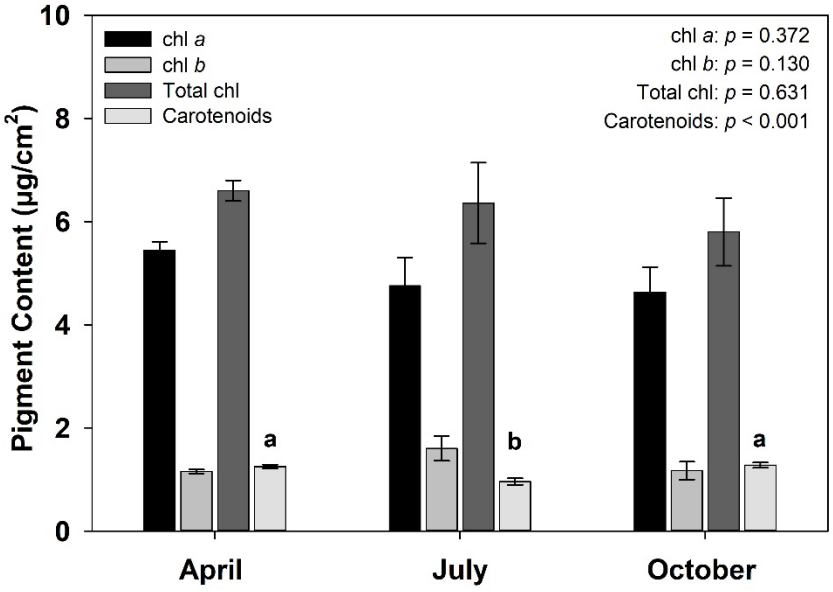

Fig. 5 Seasonal pigment concentrations for autumn olive throughout the 2017 growing season

There were no significant differences among months for leaf mass per area (Fig. 6; $p = 0.290$), which only varied from 0.0028 ± 0.0001 g cm^{-2} in July to 0.0032 ± 0.0001 g cm^{-2} in the other months.

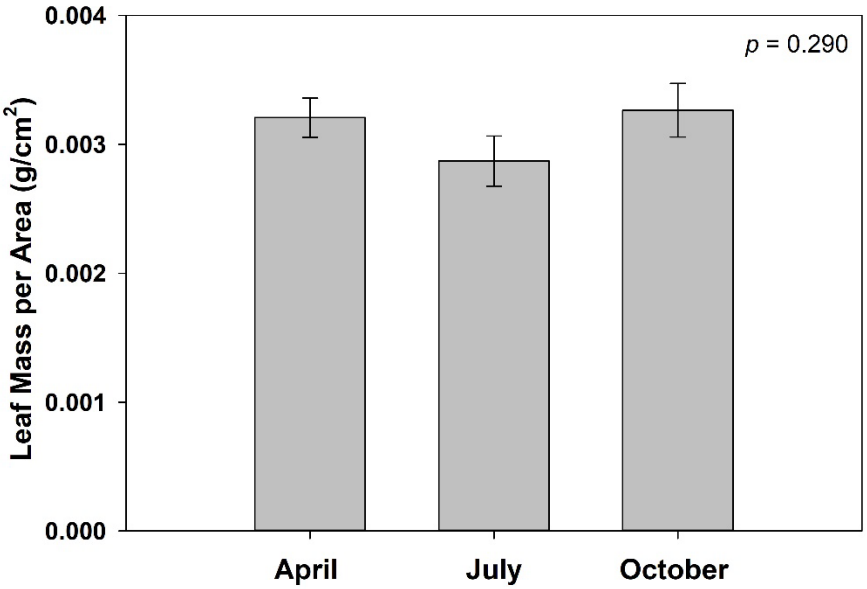

Fig. 6 Seasonal Leaf mass per area for autumn olive throughout the 2017 growing season

The C:N ratio for leaves collected in March was significantly lower ($p \leq 0.001$) than leaves collected in July, but no difference was found between the AO leaves and congeners collected in July after the pairwise multiple comparisons tests (Fig. 7A). The mean C:N ratio for March AO leaves was 9.19 ± 0.37, for July AO leaves it was 13.57 ± 0.50, and for July congeners it was 21.25 ± 2.63.

AO leaves collected in March had a significantly higher percent nitrogen content than AO leaves collected in July (4.78 $\pm$ 0.20 vs 3.38 $\pm$ 0.15%; $p \leq 0.001$). The AO leaves collected in July also had a significantly higher percent nitrogen content than the congeners (2.31 $\pm$ 0.35%) collected in July (Fig. 7B; $p \leq 0.001$).

The $\delta^{15}N$ for AO leaves collected in March (-3.74 $\pm$ 0.84 ‰) was significantly lower than in July (-1.30 $\pm$ 0.15 ‰) and the July AO leaves were significantly higher than the July congeners (-2.80 $\pm$ 0.51 ‰; $p = 0.002$), but there was no significant difference found between the March AO leaves and the July congeners (Fig. 7C).

The C:N ratio of the soil collected inside the stand of AO (15.1 ± 0.18) was significantly ($p < 0.001$) lower than the soil collected outside the stand (17.0 $\pm$ 0.37).

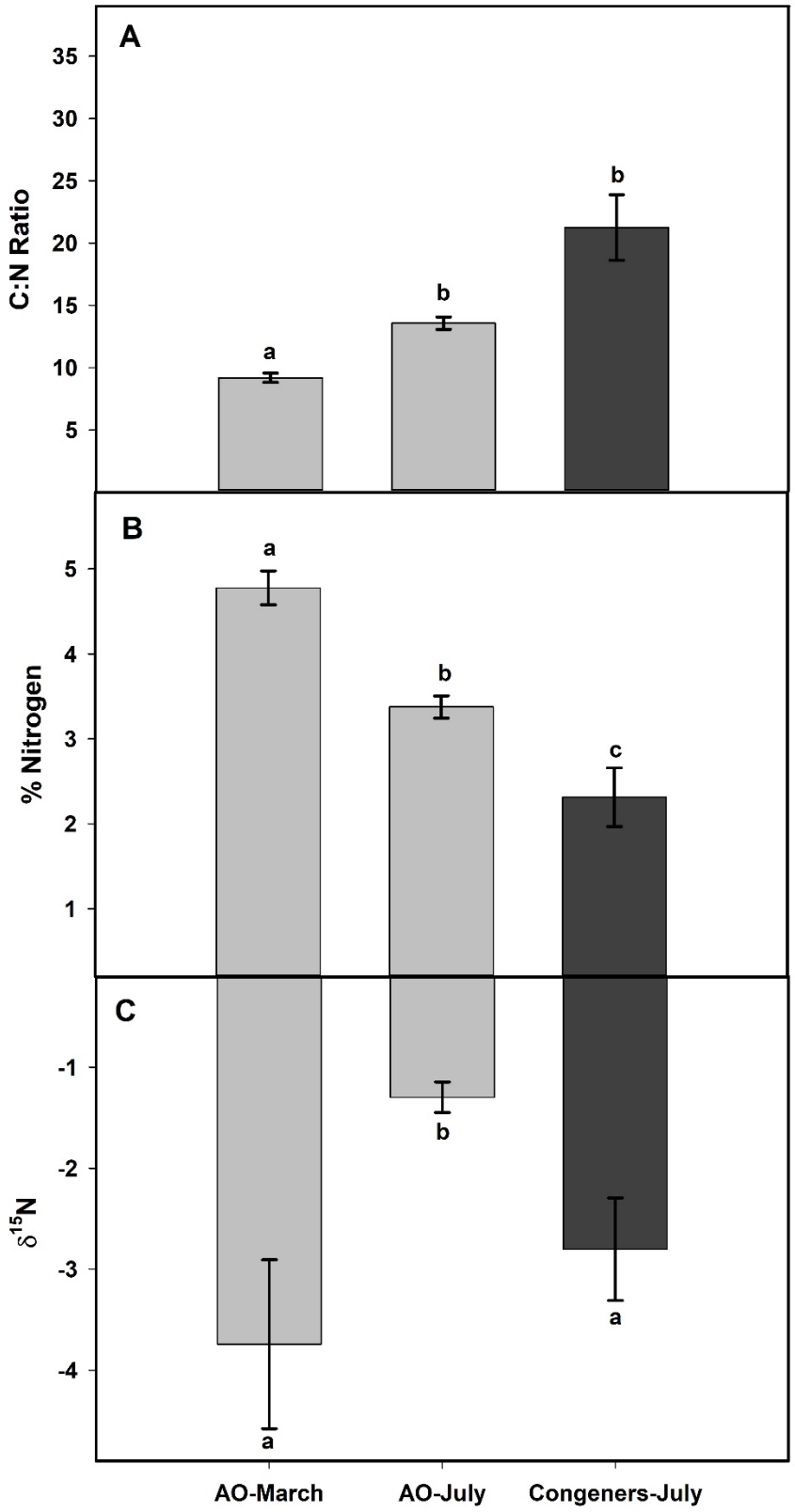

Fig. 7 Leaf nitrogen analysis completed at the Colorado Plateau Stable Isotope Lab. Autumn olive leaves for March, July, and congeners for July compared C:N Ratio (A), %N (B), and $\delta^{15}N$ (C)

Water Stress

Due to time constraints, pre-dawn water potentials were only measured during the hottest and driest portion of the season, which was in July. Pre-dawn water potentials were less than -0.1 MPa, indicating a lack of water stress at the site (Fig. 8). Daily minimum water potentials did not drop below -0.54 ± 0.04 MPa, also indicating that even in the middle of summer, AO was not experiencing much, if any, water stress.

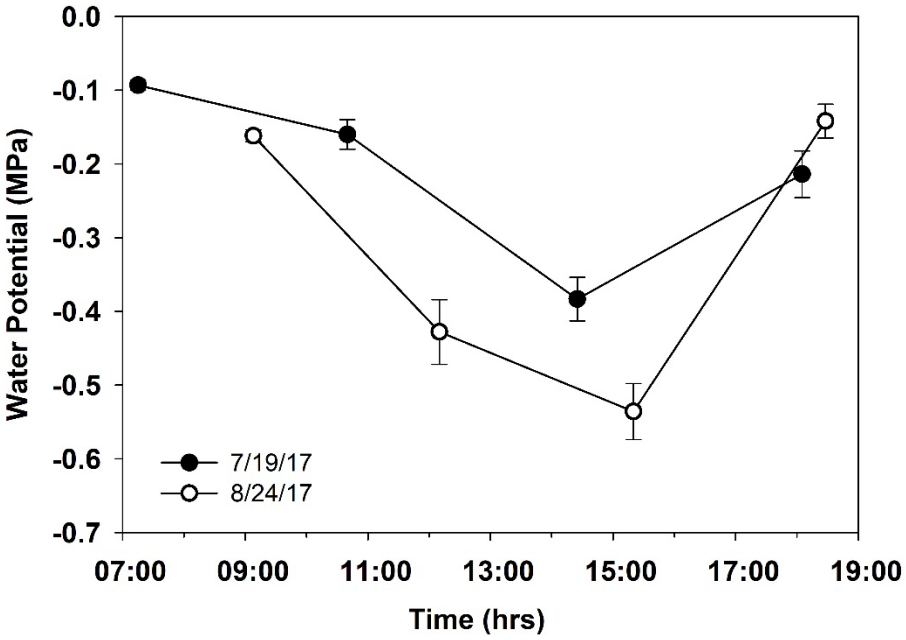

Fig. 8 Diurnal water potentials for autumn olive for the months of July and August

Gas Exchange Measurements: *Diurnals*

Temperature and PAR Seasonal Changes

Seasonal changes in light and temperature were measured during the monthly diurnal measurements (Fig. 9-15). In April, the temperature peaked at 25ºC and PAR at 1007 µmol m^{-2} s^{-1}. Both of these parameters decreased in May as the canopy started to leaf out, with maximum temperature of 21ºC and PAR of 750 µmol m^{-2} s^{-1}. The following month maximum temperature was essentially the same, but PAR dropped drastically to a maximum of just 27 µmol m^{-2} s^{-1}. July was probably the time of peak canopy cover and maximum temperature rose to 27ºC while PAR was only 50 µmol m^{-2} s^{-1}. In August, the temperature dropped to 22ºC and PAR remained low at 30 µmol m^{-2} s^{-1}, a day that also had some cloud cover. In September canopy leaf loss began towards the end of the month, and the maximum temperature was 25ºC with PAR of 40 µmol m^{-2} s^{-1}. The final month of diurnals was completed in October, which was warm and had maximum temperatures around 23ºC. However, due to canopy leaf loss, maximum PAR jumped to 500 µmol m^{-2} s^{-1}.

Seasonal Changes in Gas Exchange Rates

Photosynthetic rates (*A*) were highest in the spring months of April and May and then again in October, due to the higher light levels at those times (Fig. 9-15). Maximum rates of *A* were fairly moderate, peaking in April at just 6.0 ± 0.44 µmol m^{-2} s^{-1}, and were essentially the same in May at 5.6 ± 0.65 µmol m^{-2} s^{-1}. Rates in October were nearly as high as in the spring, peaking at

5.0 ± 0.51 µmol m^{-2} s^{-1}. The summer months, with much lower PAR, also resulted in much lower *A*, with monthly peaks never exceeding 1.8 µmol m^{-2} s^{-1} from June through September.

Stomatal conductance (g_s) was high in the spring months of April and May, peaking at 0.150 ± 0.01 mol m^{-2} s^{-1}, before dropping during the summer months to 0.089 ± 0.01 mol m^{-2} s^{-1} and never exceeding this value. It began to increase again in August to 0.157 ± 0.01 mol m^{-2} s^{-1}, and then reached its highest seasonal value in September with a rate of 0.220 ± 0.01 mol m^{-2} s^{-1}. g_s was lower in October, but still substantial at 0.131 ± 0.01 mol m^{-2} s^{-1}.

VPD did not show any major seasonal effects, but did vary diurnally, and was generally higher towards the end of the day, mainly due to daily heating. Over the growing season the maximum VPD varied from 1.06 ± 0.01 kPa in June, to its highest in July at 2.99 ± 0.02 kPa.

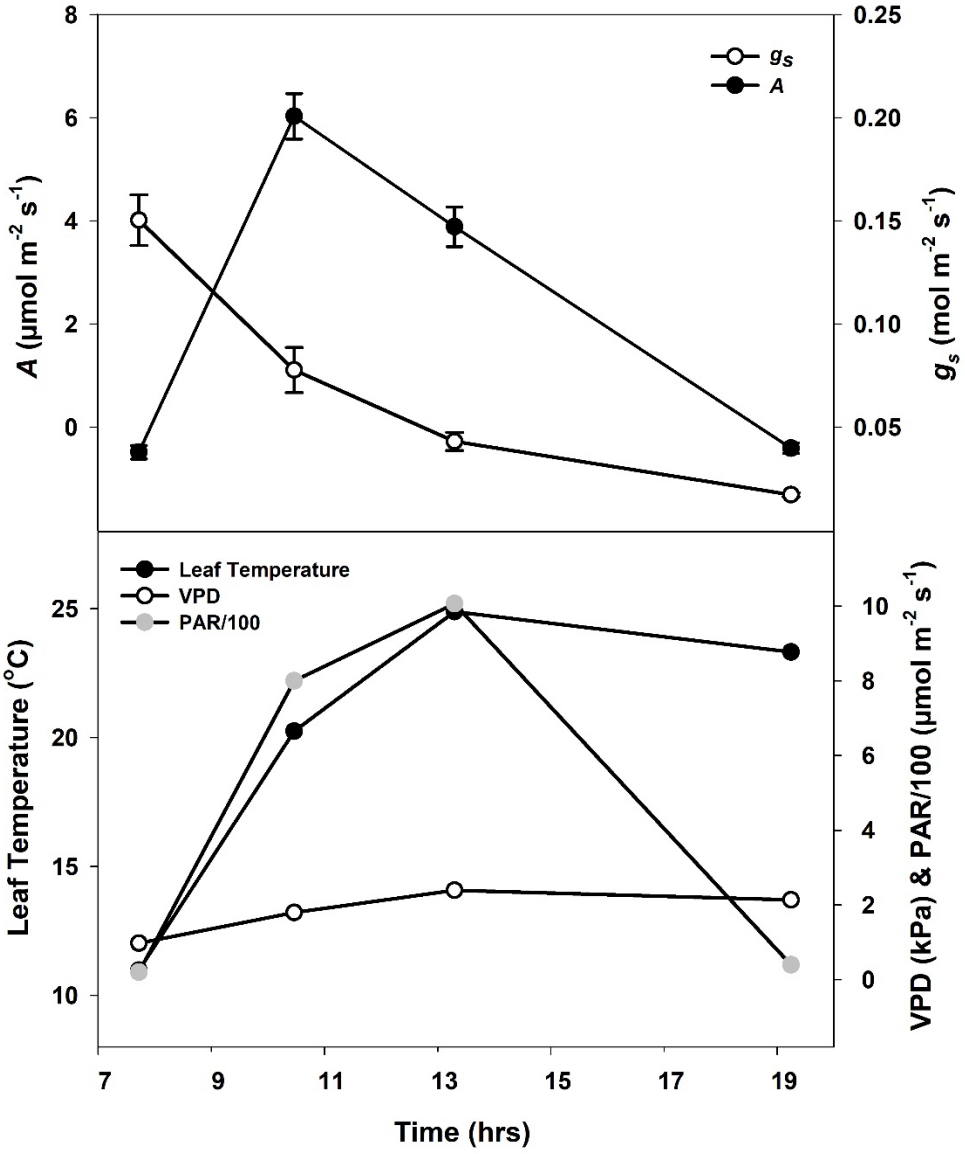

Fig. 9 Diurnal measures of photosynthesis (*A*), stomatal conductance (g_s), leaf temperature, vapor pressure deficit (VPD), and photosynthetically active radiation (PAR) for autumn olive during the month of April

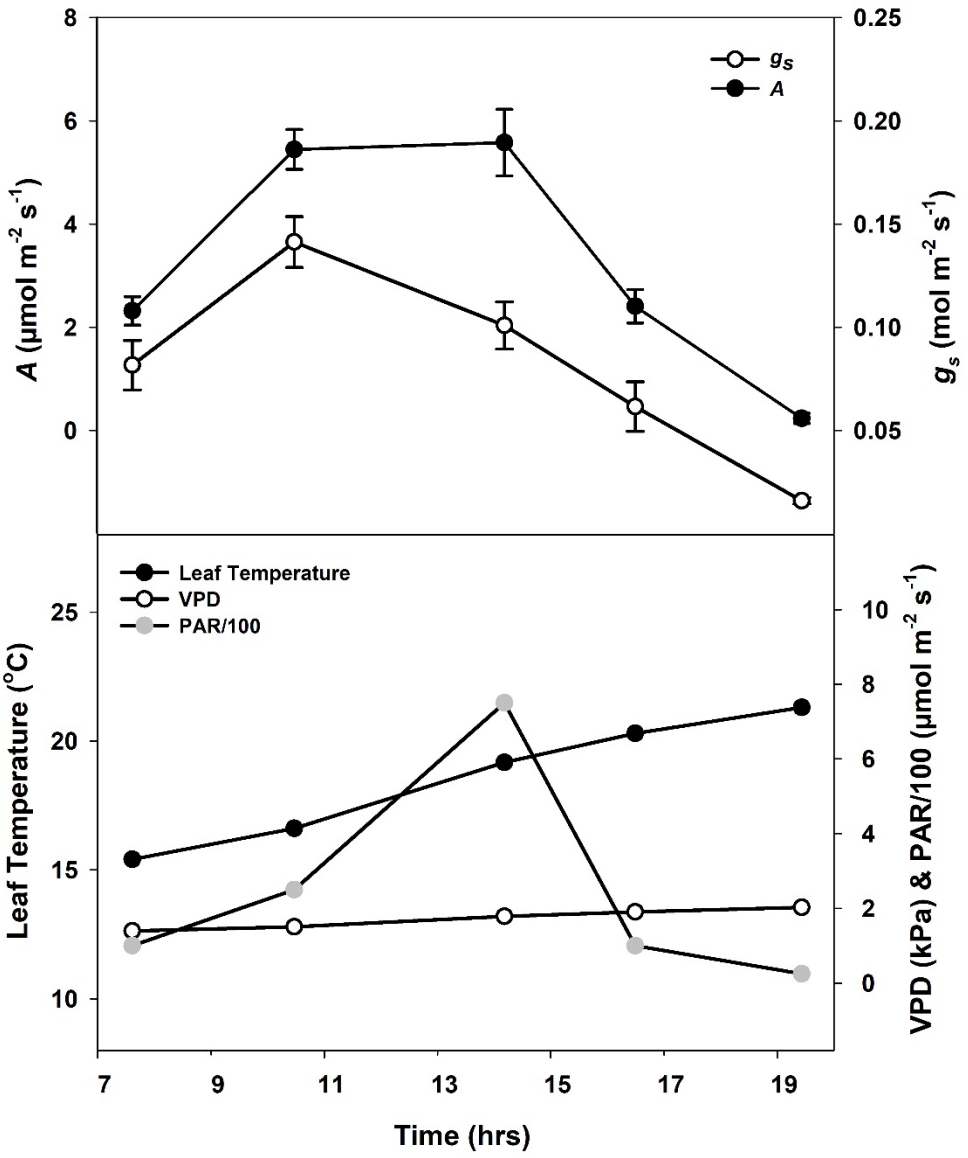

Fig. 10 Diurnal measures of photosynthesis (*A*), stomatal conductance (g_s), leaf temperature, vapor pressure deficit (VPD), and photosynthetically active radiation (PAR) for autumn olive during the month of May

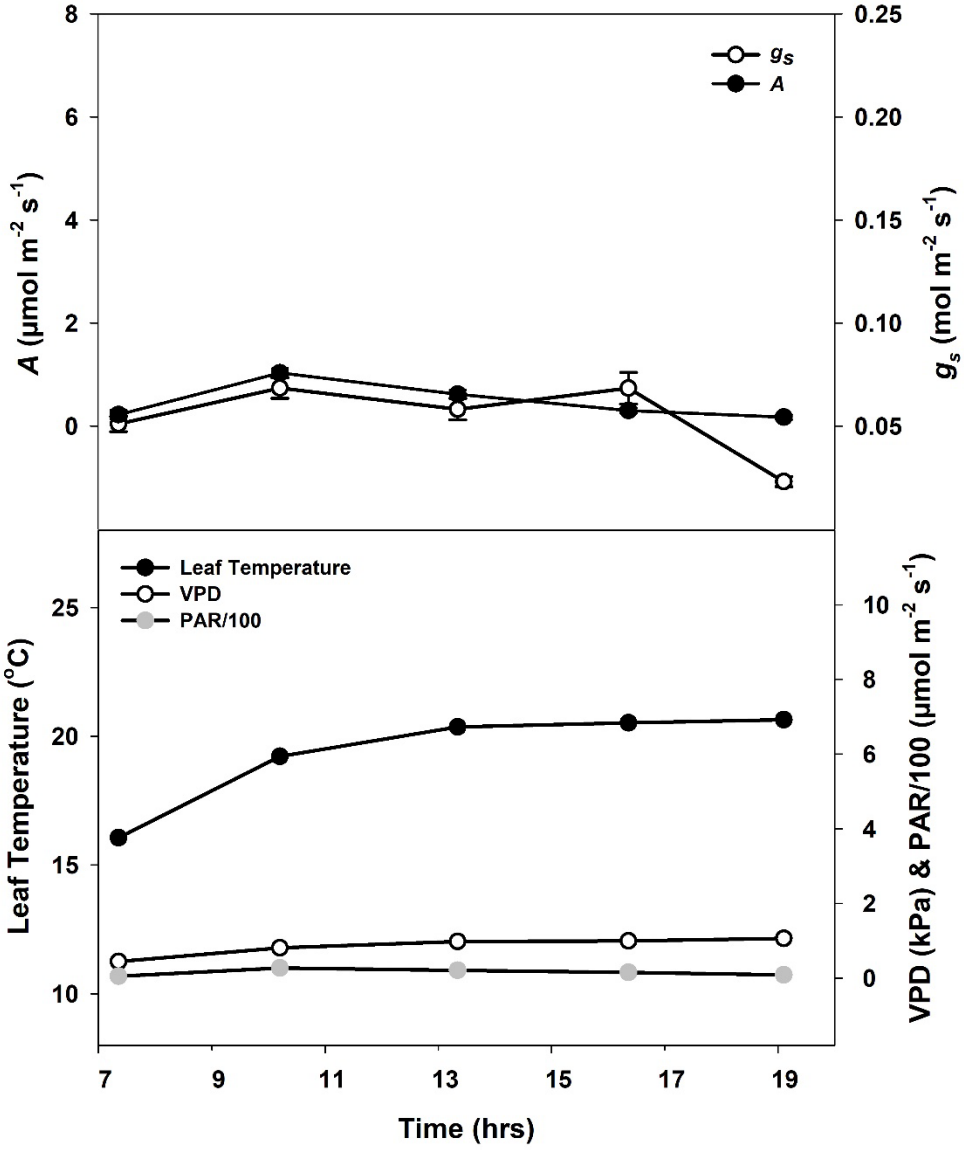

Fig. 11 Diurnal measures of photosynthesis (*A*), stomatal conductance (g_s), leaf temperature, vapor pressure deficit (VPD), and photosynthetically active radiation (PAR) for autumn olive during the month of June

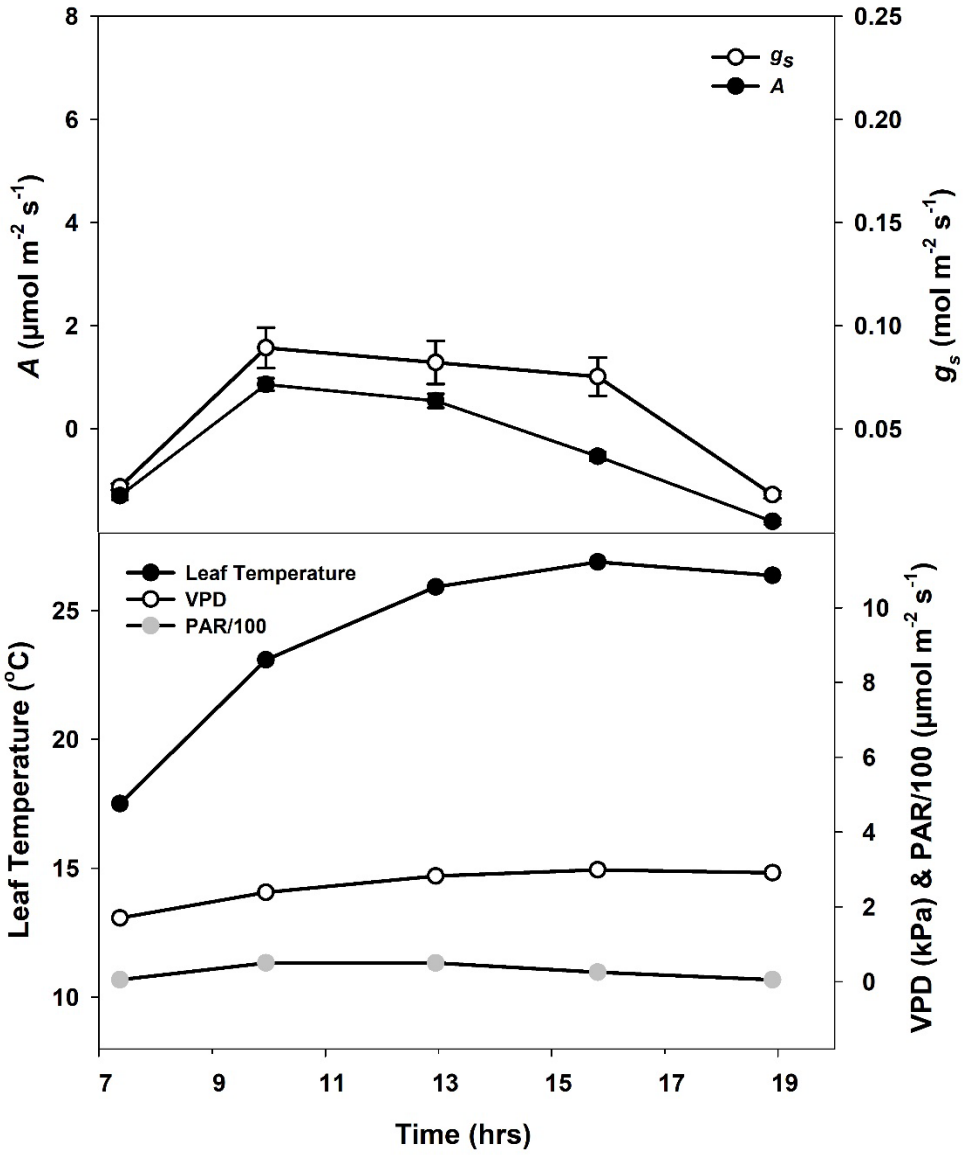

Fig. 12 Diurnal measures of photosynthesis (*A*), stomatal conductance (g_s), leaf temperature, vapor pressure deficit (VPD), and photosynthetically active radiation (PAR) for autumn olive during the month of July

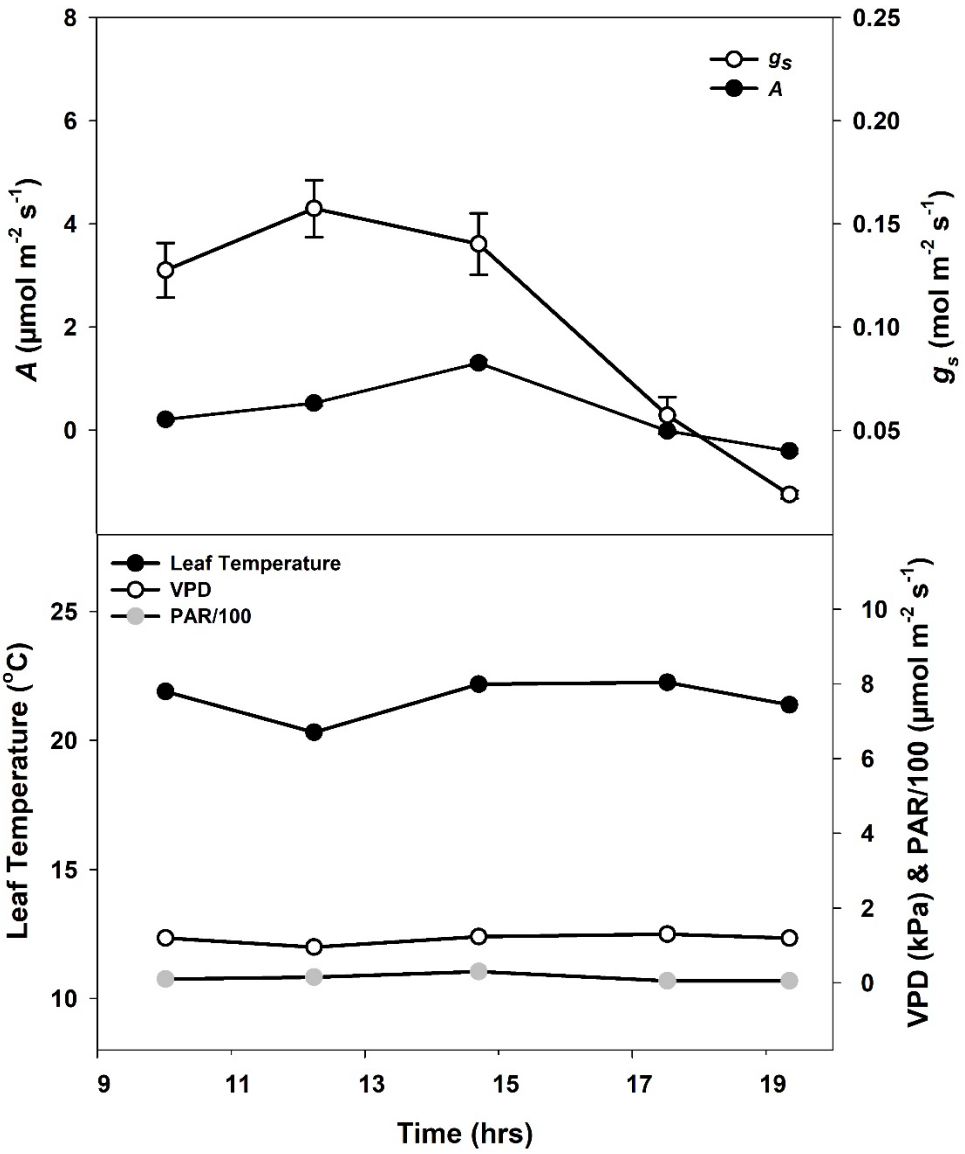

Fig. 13 Diurnal measures of photosynthesis (*A*), stomatal conductance (g_s), leaf temperature, vapor pressure deficit (VPD), and photosynthetically active radiation (PAR) for autumn olive during the month of August

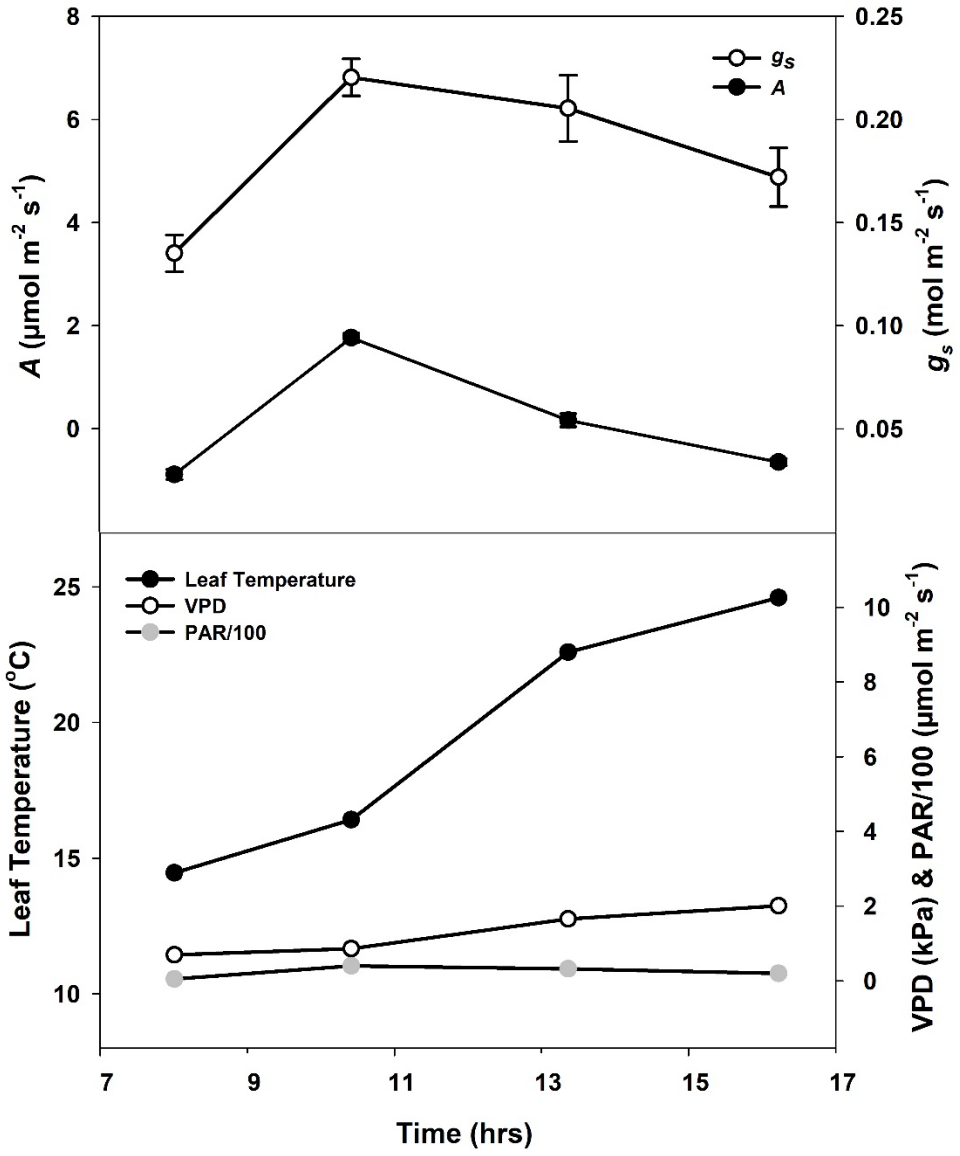

Fig. 14 Diurnal measures of photosynthesis (*A*), stomatal conductance (g_s), leaf temperature, vapor pressure deficit (VPD), and photosynthetically active radiation (PAR) for autumn olive during the month of September

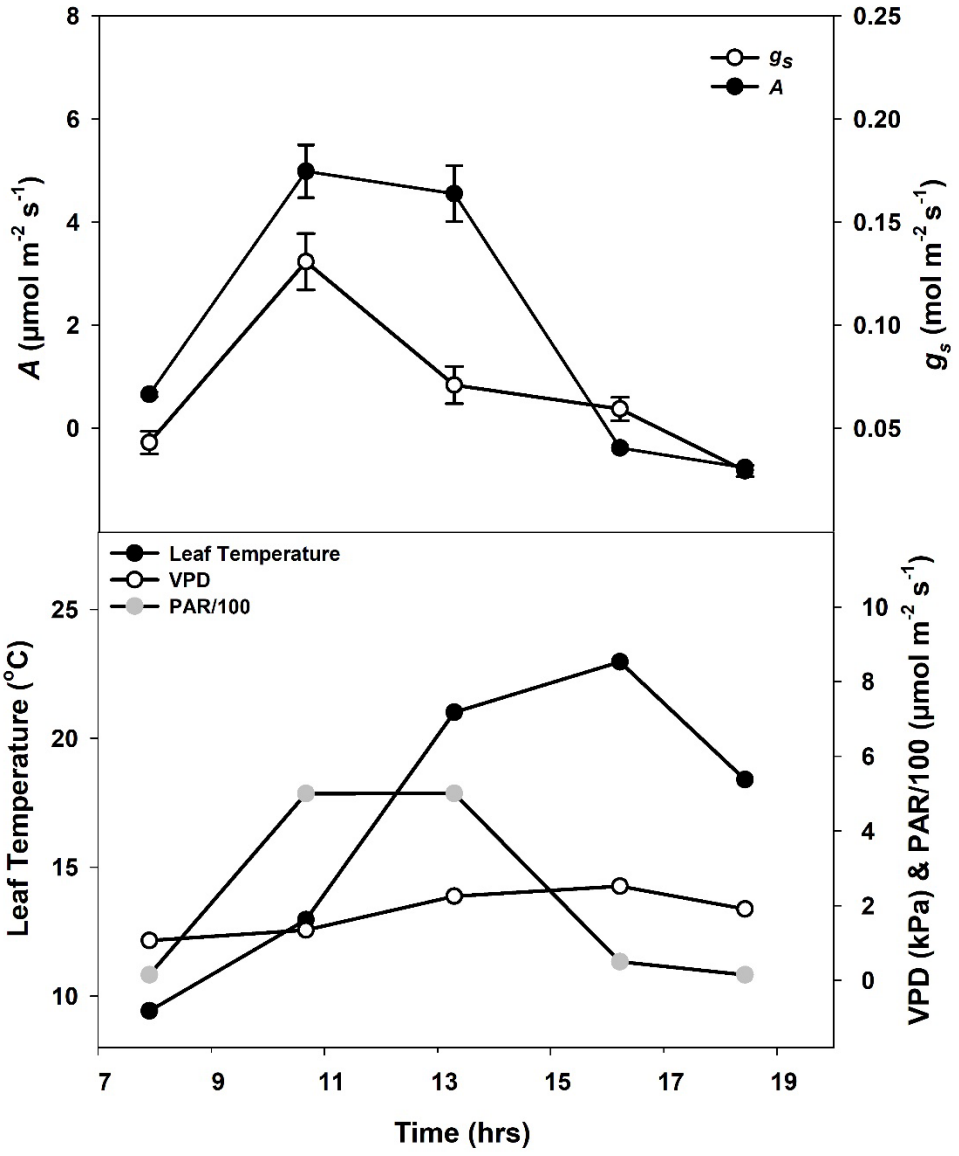

Fig. 15 Diurnal measures of photosynthesis (*A*), stomatal conductance (g_s), leaf temperature, vapor pressure deficit (VPD), and photosynthetically active radiation (PAR) for autumn olive during the month of October

Daily Carbon Gain

Integrating the diurnal assimilation curves showed that AO gained the majority of its carbon on a per leaf basis in April and May with rates of 127 ± 14.1 mmol m^{-2} day^{-1} and 161 ± 22.9 mmol m^{-2} day^{-1}, respectively. These gains occurred before the overstory canopy was fully leafed out and when AO had access to high light (Fig. 16). There was minimal carbon gain through the summer months (June-September) and some days actually had negative accumulation rates, e.g., July (-7 ± 6.4 mmol m^{-2} day^{-1}) due to the extremely low light levels. In October AO was again able to achieve a higher, positive carbon gain of 91 ± 12.2 mmol m^{-2} day^{-1} because the canopy had partially re-opened and PAR was higher.

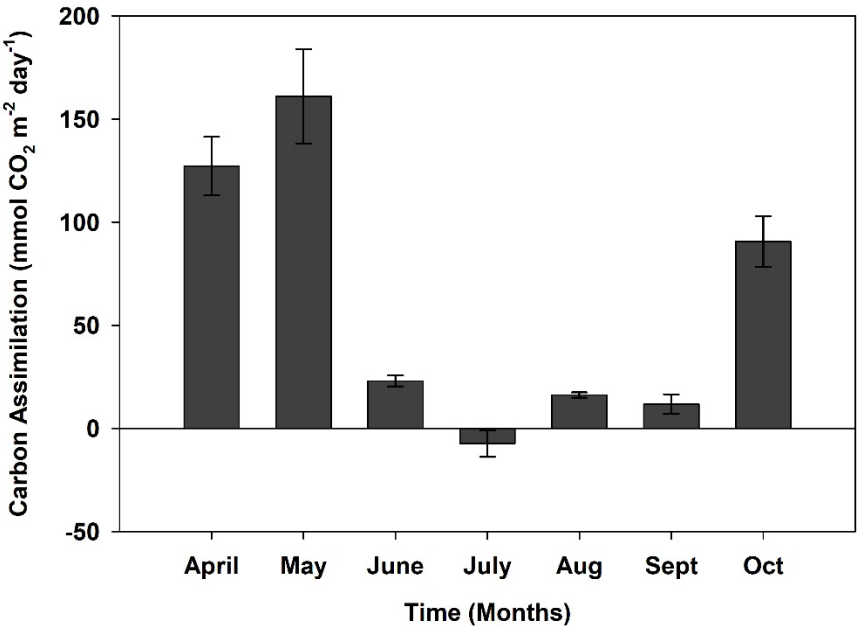

Fig. 16 Daily carbon gain, on a per leaf basis, for autumn olive throughout the growing season of 2017

Environmental Response Curves for Gas Exchange

Light Response Curves

All light curves were fitted with a 3-parameter exponential rise to maximum equation and the means of each parameter can be found in Table 1, along with the results of the post-hoc tests. The monthly comparison of light curves shows that A_{max} was much lower in July (5.56 ± 0.81 µmol m^{-2} s^{-1}; $p = 0.006$) compared to other months (9.72 $\pm$ 0.93 µmol m^{-2} s^{-1}), even though each plant received the same amount of light (Fig. 17A). Other parameters were also significantly lower in July, including: dark respiration ($p < 0.001$), Light Compensation Point ($p = 0.002$), and Saturation Light Intensity ($p = 0.006$). Apparent Quantum Efficiency was significantly lower ($p = 0.031$) in July than October and water use efficiency (A/g_s) was significantly lower ($p < 0.001$) in October compared to April and July (Table 2), because of higher g_s in October (Fig. 17D).

Stomatal conductance showed similar amounts of increase with increases in light for the months of April and October, however the maximum rates for April were 0.111 ± 0.03 mol m^{-2} s^{-1} and for October they were more than double at 0.274 ± 0.01 mol m^{-2} s^{-1}. In July the plants showed little to no change in stomatal conductance with change in light, and the averages were lower than seen in April (Fig. 17D).

Temperature Response Curves

Temperature curves were fitted with 3 parameter 2nd degree polynomial functions and the means of each parameter can be found in Table 1. *A* increased with temperature until it reached an optimum for each month and then declined

again. Photosynthetic rates showed a temperature optimum of 25°C for April and October, but 30°C in July. The temperature response of g_s was minimal for the months of May and July and the rates were low for these months as well (Fig. 17E), however in October g_s peaked at 20°C with a rate of 0.246 ± 0.05 mol m^{-2} s^{-1} before dropping as temperature was further increased.

VPD Response Curves

VPD curves were fitted with 3 parameter 2nd degree polynomial functions and the means of each parameter can be found in Table 1. Stomatal conductance was most responsive to VPD in May, dropping by 50% (from 0.218 mol m^{-2} s^{-1} to 0.108 mol m^{-2} s^{-1}) as VPD increased from 0.99 to 2.94 kPa. Conductances were less responsive to VPD in July and October. In July g_s started at 0.112 mol m^{-2} s^{-1} at a VPD of 0.99 kPa before peaking at 0.140 mol m^{-2} s^{-1} at a VPD of 2.0 kPa. g_s then dropped with increasing VPD. In October, the g_s was lower than in May, but it dropped by 70% (from 0.161 mol m^{-2} s^{-1} to 0.114 mol m^{-2} s^{-1}) from a VPD of 1.50 to 2.90 kPa.

A showed similar trends to g_s, and in May and October significantly decreased with increasing VPD. In May the response was almost linear, dropping 77% (12.4 µmol m^{-2} s^{-1} to 9.6 µmol m^{-2} s^{-1}) as VPD increased from 0.99 kPa to 2.94 kPa. In July photosynthetic rates averaged 9.6 µmol m^{-2} s^{-1} with minimal change as VPD increased.

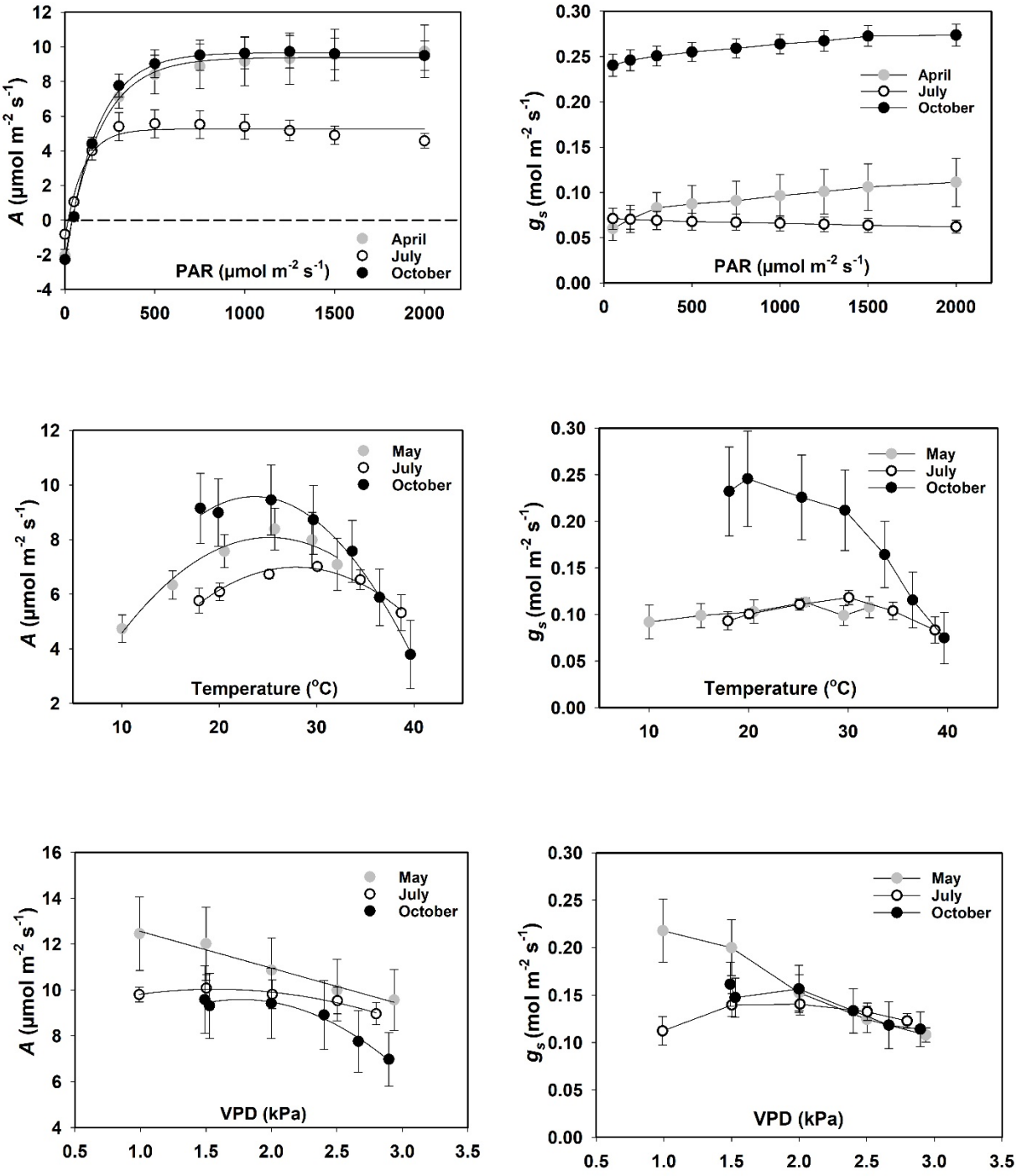

Fig. 17 Seasonal response curves for autumn olive, including light (A,D), temperature (B,E), and VPD (C,F). The photosynthetic rates (*A*) are shown on the left side (A-C) and the stomatal conductances (g_s) are shown on the right side (D-F)

Table 1. Parameters used for regressions in response curves, gathered using Sigma Plot. Equations for each curve can be found in the methods.

Light	y_0	a	b
April	-2.1847	11.5725	0.0053
July	-1.0185	6.2702	0.0101
October	-2.5013	12.1713	0.0057

Temperature	y_0	a	b
April	-1.5618	0.7657	-0.0152
July	-3.4789	0.7508	-0.0135
October	-2.8007	1.0483	-0.0222

VPD	y_0	a	b
April	-14.1696	-1.6215	0.0061
July	8.3563	2.1354	-0.6804
October	3.0742	7.365	-2.0824

Table 2. Monthly comparison of light curve parameters. Values are mean ± se, n=5.

Light Curve Parameters	April	July	October
Dark respiration ($\mu mol\ m^{-2}\ s^{-1}$)	-2.050 ± 0.347^{a}	-0.826 ± 0.109^{b}	-2.275 ± 0.180^{a}
Light Compensation Point ($\mu mol\ m^{-2}\ s^{-1}$)	46.646 ± 5.561^{a}	22.454 ± 3.066^{b}	46.482 ± 4.380^{a}
Apparent Quantum Efficiency ($\mu mol\ CO_2 / \mu mol_{photons}$)	0.043 ± 0.003^{ab}	0.032 ± 0.004^{a}	0.044 ± 0.002^{b}
Amax ($\mu mol\ m^{-2}\ s^{-1}$)	9.439 ± 1.471^{a}	5.003 ± 0.564^{b}	9.612 ± 0.898^{a}
Saturation Light Intensity ($\mu mol\ m^{-2}\ s^{-1}$)	9.156 ± 1.427^{a}	4.853 ± 0.548^{b}	9.324 ± 0.871^{a}
Water Use Efficiency ($\mu mol\ CO_2 / mol_{H2O}$)	95.144 ± 8.341^{a}	79.059 ± 6.903^{a}	35.983 ± 4.085^{b}

Discussion

The results of this study show that AO is capable of surviving in the understory of a southern Appalachian forest, primarily by taking advantage of an extended leaf phenology and its symbiotic relationship with bacteria that fix nitrogen. These two traits confer a competitive advantage to AO that allows it to establish, grow, and eventually dominate the understory, even though AO is normally associated with open-field habitats. The ability to reproduce vegetatively may also contribute to its ability to dominate in this particular habitat.

Climate and Phenology

Autumn olive thrives in open field environments both in its native habitat as well as its non-native habitat here in the U.S. (Ahmad et al. 2006; Brym et al. 2014). Because of this dominance in open-field habitats, it has been classified as shade intolerant (Catling et al. 1997; Ahmad et al. 2006; Brym et al. 2014). However, AO has been found in habitats with a wide range of light availability, suggesting that it has physiological mechanisms that allow it to extend its niche into low light environments (Brym et al. 2011; Zinnert et al. 2013; Shiflett et al. 2017). Brym et al. (2014) found that AO seedlings were more abundant under the canopy in a low light environment than open environments and that these seedlings were probably more limited by water availability than light. In contrast, adult plants were more limited by neighborhood competition coupled with light availability. They concluded that AO would do best in edge environments, where there is an intermediate amount of light and slightly reduced competition.

At my study site in the ASU Nature Preserve, AO was located only in the interior of the forest, under a dense canopy. In such a closed canopy system, light availability is often the main driver of plant dynamics (Pacala et al. 1996; Kobe 1999; Brym et al. 2011, Neufeld and Young 2014). In this situation, AO only has access to high light in the spring and fall when the canopy is leafless, with the exception, of course, when there are sunflecks (Chazdon 1988). Sunflecks can be disproportionally important for carbon gain in understory plants (Neufeld and Young 2014) but were not the focus of the research described herein. Nonetheless, they could be important to the persistence of AO in understory habitats, and should be the subject of future research efforts. Hemispherical photographs of the canopy in April and October showed that the percent of open sky was significantly higher in those months than during July, and most importantly, during those high light times, AO was leafed out and could take advantage of those high PAR values.

Autumn olive bud break began on February 14th in 2017 and during the next two months no woody native species were leafed out. Other invasives such as *multiflora rose and barberry* also leafed out at similar times as AO; in fact, *R. multiflora* was often the first to leaf out with *B. thunbergii* the last of these three woody invasives to do so. In early April, spring wildflowers began leafing out, such as *Viola* sp., but no woody congeners that compete with AO were leafed out at this time. This could be due to the temperatures n February, which averaged 6°C in Boone, NC. AO completed leaf out just as the canopy began to leaf out, which allowed high light to reach the understory through April 8th, ~53 days after leaf out began for AO. This means that AO had nearly two months to take advantage of the

relatively high light, which would give it a competitive advantage over native congeners.

My results differed some from the study done by Fridley (2012). Fridley found that woody invasives also had a significantly longer phenology, but he concluded that in upstate New York, where his study was conducted, the invasives benefited more in the fall than the congeners, while differences in their spring phenological extension were insignificantly different from native congeners. In the ASU Nature Preserve, I observed that AO extended its phenology at both ends of the growing season and that it was extended longer in the spring than in the fall. Fridley's study was done in Syracuse, NY while my work was located in the mountains of western NC. This suggested to me that a temperature difference between these regions might have been the cause of this timing difference. Using archived weather data from the NOAA website (https://www.ncdc.noaa.gov/cdo-web/datasets) I averaged the spring (March, April, and May) temperatures for the last 10 years for Boone, NC and Syracuse, NY, only to find that the two regions differed by less than 2°C, with Boone being warmer. The difference in temperature could be a factor allowing AO to leaf out earlier in the south, but the small magnitude of the difference suggests that it may not be the sole factor distinguishing the two sites, and that another factor, such as latitude, might play a role. Northern habitats would have significantly shorter days in the early spring and late fall, and a lower solar angle, than sites in the south, and these two factors could possibly affect the timing of leaf out.

Species are known to respond to the combination of photoperiod and temperature (Flynn and Wolkovich 2018) and different species have different

requirements for leafing out in the spring. The three main factors affecting leaf out for woody species are (1) chilling requirements, (2) spring temperature (both air and soil), and (3) photoperiod (Farmer 1968; Ghelardini et al. 2010; Polgar and Primack 2011; Flynn and Wolkovich 2018). With climate change it is likely that warmer temperatures, which cue spring leaf out for some species, will begin to occur earlier in some regions (Schwartz and Reiter 2000; Parmesan 2006; Ibáñez et al. 2010). If however, photoperiod is the main requirement, then species will not leaf out earlier in response to increased temperature, but may exhibit more rapid leaf growth once they do leaf out (Bull 1968; Monsi and Murata 1970; Gallagher 1979). However, late leafing species could miss out on potential carbon gain in the early spring, and be at a competitive disadvantage (Fridley 2012). Zhang et al. (2007) discussed how species growing at different latitudes will experience different chilling requirements and increased temperatures with the onset of climate change. Species located in higher latitudes (above 40°N) are suspected to still achieve successful chilling requirements whereas below 30°N temperatures may become so warm that species won't have successful chilling requirements (Zhang et al. 2007). This climate fluctuation can allow for earlier leaf out times if the species is relying on temperature cues and in the right area, or if they do not have chilling requirements (Linkosalo et al. 2006; Polgar and Primack 2011).

If a species is relying on an extended leaf phenology and able to have extensive leaf growth before native congeneric species, then they will be able to gain carbon early in the spring (Polgar and Primack 2011). Long-lived trees are more likely to have photoperiod requirements (Caffarra and Donnelly 2010; Polgar and

Primack 2011), while short-lived early successional species are more likely to have chilling and/or temperature requirements (Körner and Basler 2010; Polgar and Primack 2011). Many invasives have extended leaf phenologies and would have the potential to leaf out even earlier as the climate changes. Autumn olive behaves like an opportunistic, early-successional species and therefore may likely take advantage of these climatic shifts which could exacerbate its invasiveness.

AO is known for its abundant fruit production in open-field habitats (Fordham et al. 2001; McCall and Walck 2014), and the seeds are primarily distributed by birds (Bonilla and Pringle 2015). Fruit production requires a significant investment of energy, particularly in the form of non-structural carbohydrates and proteins, and in low light environments, such as a forest understory, the availability of these resources would be limited. I noticed that in my study site, AO produced very few flowers and fruits throughout the season (Riffe, personal observation). The diurnal gas exchange patterns showed that carbon gain by AO in the summer, when the canopy is fully leafed out, is marginal at best, and would probably not provide enough photosynthates for fruit and seed production. This means that AO most likely reproduces and spreads through vegetative propagation. This species is a prolific sprouter, and can spread by root sprouts, much as do beech trees (*Fagus americana*) (Szafoni 1991; McCall and Walck 2014). Thus, the ability to persist and spread may be facilitated by its switch from sexual to asexual reproduction in the understory. This has implications for genetic diversity in understory patches of AO, and would be a subject worthy of future study to determine the relatedness of individuals and the genetic diversity of individuals within understory patches.

Leaf pigments, Leaf Mass per Area, Nitrogen

Certain leaf characteristics such as leaf pigment concentrations and leaf mass per area are indicative of a plant's photosynthetic productivity (Reich et al. 1998; Wright et al. 2004; Zinnert et al. 2013) so it is important to look at leaf characteristics of AO in both open (Zinnert et al. 2013) and understory environments (Brym et al. 2011; this study). AO has characteristics that allow for extensive light capture and a high photosynthetic capacity (high %N, high leaf mass per area in the sun), both of which are characteristics of shade-intolerant plants (Brym et al. 2011; Zinnert et al. 2013). In open habitats, AO can have over 4x the chl amounts compared to what I found in the understory (Zinnert et al. 2013), which no doubt contributes to its ability to harvest the high light levels found in open habitats. The leaf mass per area (LMA) for my understory AO plants is lower than that of leaves from open environment plants, but similar to what Brym et al. (2011)[1] found for AO in the understory. This finding is consistent with known sun-shade differences in leaf anatomy (Neufeld and Young 2014). Interestingly, this suggests that light levels are not high enough in the understory to produce sun leaves even when AO leaves flush beneath an open canopy. There are several likely reasons for this. First, light levels in the spring may not be high enough to elicit a sun-leaf anatomy, even though they can reach rates above 1300 µmol m^{-2} s^{-1} beneath the leafless canopy. Second, it could be due to the short day lengths at this time of the year. Nobel and Hartsock (1981) found that it was the integrated total radiation received that determined

[1] In Figure 2 in Brym (2011) the units for specific leaf area are cm^2 per mg, which does not yield reasonable values; I believe the mg should be g.

whether a plant formed a sun- or shade-leaf anatomy. Long days with low instantaneous PAR could induce a sun-leaf anatomy as could short days with high instantaneous PAR. Thus, for AO, the combination of short days and moderate PAR in the early spring may induce a shade-leaf anatomy in this species.

Nitrogen content is an important factor in plant invasiveness and plant competition (Díaz-Barradas et al. 2015) because high N amounts allow for higher rates of photosynthesis (Dornbos et al. 2016). Nitrogen fixing species can be distinguished from non-fixing species on the basis of their higher leaf N (Cohen et al. 1980). AO is known to fix nitrogen when growing in open habitats, but little is known about its ability to carry on this process in the understory, since fixation is a very energetically expensive process and there may not be sufficient light in the understory to support this process. One possibility is that AO only fixes nitrogen in the early spring when it has access to high light. However, fixation at this time of the year could be limited by cold soil temperatures (Jones and Tisdale 1921; Hardy et al. 1968; Roughley and Date 1986; Legros and Smith 1994; Lynch and Smith 1994; Bordeleau and Prévost 1994; Zhang et al. 1996) and AO may only achieve high rates later in the spring once soils have heated up.

I employed four different methods to determine if AO plants in the understory were fixing nitrogen (leaf C:N, %N in the leaf, $\delta^{15}N$ in the leaf, and soil C:N). If AO is fixing nitrogen then the C:N ratio in leaves and soil (Funk et al. 1979) should be lower, and the %N higher, than in congeners that don't fix N. Furthermore, the $\delta^{15}N$ value in AO leaves should be less negative than non-fixing congeners, because they

are relying more on atmospheric nitrogen, which has a $\delta^{15}N$ of ~0‰ (Unkovich 2013; Craine et al. 2015).

Three traits (C:N, %N, and soil C:N) showed significant differences from the non-fixing congeners. They followed patterns that would suggest AO is or has carried out N-fixation: the C:N ratios in the leaves and soil were significantly lower, and the %N is higher in AO than for the congeners. The C:N ratio for my AO leaves collected in March more closely matched that of understory leaves from Brym et al. (2011) while the leaves collected in July more closely matched open environment leaves from Zinnert et al. (2013). For percent nitrogen, both of my AO collections were close to Zinnert's (open environment) values for AO but significantly higher than Brym's (understory) leaves. However, my leaves had a lower LMA, meaning they were thinner and wider than Zinnert's leaves. This means that on a per area basis my leaves would have less nitrogen content, which could explain the lower rates of photosynthesis on a per leaf area basis that we saw throughout the year (Reich et al. 1998).

Considering that the C:N ratio for AO leaves increased from March to July, but the leaf mass per area remained unchanged seasonally it is likely that the change is solely due to the decrease in %N. This decrease in leaf nitrogen during July might be due to a decrease in RUBISCO during the summer months and perhaps translocation of the released N to other plant parts. The lack of change in leaf mass per area argues against a dilution effect because of continued leaf growth.

However, the $\delta^{15}N$ values for AO leaves collected in March (when the AO had high light and could potentially be fixing N) were not significantly different from the

congeners collected in July, while the AO leaves collected in July were significantly higher than the March AO or congener samples (Fig. 7C). A higher value (closer to zero) for $\delta^{15}N$ than the native species would indicate fixation (Spriggs et al. 2003; Hobbie and Hobbie 2008; Brym et al. 2011). The results could be interpreted to mean that AO had not yet started to fix nitrogen when I sampled the leaves in March, perhaps because soils were cold, and nodulation had yet to occur for that growing season (Jones and Tisdale 1921; Hardy et al. 1968; Roughley and Date 1986; Legros and Smith 1994; Lynch and Smith 1994; Bordeleau and Prévost 1994; Zhang et al. 1996), but by the time I sampled in July, the soils had warmed, nodules had formed, and fixation had occurred during the months of April and May, when the canopy was not yet leafed out. Thus, the July measurements may reflect fixation activity that actually occurred several months earlier when the light levels were higher.

Another factor that can affect $\delta^{15}N$ is the degree to which trees are associated with mycorrhizal fungi (Robinson 2001), which can result in more negative values (Spriggs et al. 2003). It should be noted that there are also quality control and processing issues which can lead to inconsistent $\delta^{15}N$ values (Unkovich 2013) and so any results should be interpreted with caution.

In summary, the suite of data from the four N abundance and ratio measures strongly suggest that AO is capable of fixing N in the understory. However, maximum rates of photosynthesis are not very different from those obtained from native species in similar habitats (Sullivan et al. 1996), and so the question of how high leaf N benefits AO is an open question. One possibility is that the high leaf N

content enables AO to synthesize larger quantities of RUBISCO, the carboxylation enzyme, which usually constitutes the largest sink for leaf N (Sharkey et al. 1991), and that in turn, allows the leaf to photosynthesize in early spring and fall when light levels are high, and for the plant to produce large numbers of leaves that can shade out other plants. By distributing the N among its many leaves, it can assimilate large amounts of carbon and grow rapidly, even when the maximum rate of photosynthesis is unremarkable compared to congeneric native species. Lastly, it is unclear if AO carries out N fixation once the canopy is fully leafed out, and determining this would require more detailed physiological studies involving sampling for active nodules on a seasonal basis, and perhaps performing *in situ* analyses of fixation using the acetylene reduction technique (Boring and Swank 1984).

Water Stress

AO has been described as a drought tolerant species (Naumann et al. 2010), but at my study site AO primarily exists at lower, and presumably wetter areas of the preserve, which suggests that it might, in fact, be sensitive to water stress. AO has been found to have a higher specific conductivity (conductivity per unit stem area) than native species, but it also loses conductivity at a higher water potential in an open light environment (Zinnert et al. 2013). This suggests a safety vs efficiency trade-off for this species, wherein the ability to conduct water more easily is sacrificed for the avoidance of cavitation. This suggests that avoidance of severe water stress may be more important for AO than the ability to maintain water flow to

the leaves in the face of water limitations. To do this, AO would have to grow in habitats that rarely experience severe soil water deficits, and this could explain why it prefers moister habitats, such as forest coves. My water potential measurements, collected during July and August, the warmest and driest months, showed that AO plants at my site exhibited little if any water stress. Mid-day water potentials in the understory at my site were much less negative than those found by Naumann et al. (2010) during the months of June, July, and August for plants growing in open environments.

Leaves of AO in the understory are subject to low light and lower evaporative demand. The low, diffuse light incident most of the time on the leaves would only minimally affect leaf temperature, and that, coupled with the higher relative humidity (RH) in the understory compared to that in an open habitat (Geiger 1950), would reduce the VPD and lower the evaporative demand. In addition, leaves in the understory would have larger boundary layer resistances because of lower wind velocities near the ground (Geiger 1950) and this would further reduce the loss of water from leaves. Finally, low light in the understory would also reduce g_s and that would further contribute to reduced water loss from leaves in the understory. Thus, it is clear that the loss of water from AO leaves in the understory would be lower than that from leaves in open habitats, and would limit the amount of water stress that these plants experience. The lack of water stress in southern Appalachian forest understory environments could be a factor contributing to its ability to persist in these habitats and to dominate native congeners.

Gas Exchange Measurements

Monthly diurnal measurements of gas exchange were used to assess seasonal changes in the ecophysiology of this species while inhabiting the understory of a southern Appalachian forest. Although temperature can vary greatly from day to day, and can range from below to above freezing in the spring and fall, its daily maximum varied by only 6°C (from 21 to 27°C) across the monthly diurnal measurements I made in 2017. Temperature curves for this species (Fig. 17B) show only moderate changes in *A* from 20°C to 30°C. For example, in May rates of A varied from 7.6 µmol m^{-2} s^{-1} at 21°C to 8.0 µmol m^{-2} s^{-1} at 30°C, thus within the range 21 to 27°C, *A* would have been within 90% of its maximum rate in any particularly month. The g_s showed also minimal change over this temperature range, except for the month of October where stomatal conductance decreased drastically with increased temperature. Thus it can be suggested that any differences in the maximal diurnal gas exchange rates I measured were only slightly influenced by temperature differences. This does not mean that diurnal rates could not be affected by temperature, but rather, when comparing the diurnals from 2017, all of which were obtained with a restricted temperature range, that differences in maximum *A* or in g_s were most likely due to other environmental factors, most notably, PAR and perhaps VPD.

VPD can have major effects on g_s, as seen in the VPD curves for months May and October (Fig. 17F), where increases in VPD resulted in sharp drops in g_s. VPD changes with leaf temperature, so in the months when the canopy is open it is likely that VPD increases would result in lower g_s. For example, in May there was a

decrease of 77% as VPD increased from 1.0 kPa to 3.0 kPa. Lower g_s would cause diffusional limitations on *A* and a decrease from 12.5 µmol m^{-2} s^{-1} to 9.6 µmol m^{-2} s^{-1} (Fig. 17C) was subsequently observed. There was less of a VPD effect on g_s in July, because VPD was lower and the low light levels may have decreased g_s so much that it was insensitive to VPD.

Light was extremely variable in the understory, ranging from a daily maximum of 1007 µmol m^{-2} s^{-1} in April to just 27 µmol m^{-2} s^{-1} in June. Daily maximum PAR levels under 50 µmol m^{-2} s^{-1} were consistent throughout the months of June to September while the canopy was leafed out. Net photosynthesis was highest during the spring when AO was receiving the highest light, but the rates were moderate, possibly due to the fact that leaves were still young and maturing and had not yet reached their full photosynthetic potential. Naumann et al. (2010) completed diurnals on AO plants in an open field environment in coastal Virginia during the months of May to August and found maximum rates of *A* between 15 and 25 µmol CO_2 m^{-2} s^{-1}. These rates are 2 to 3 times higher than my maximum rates, no doubt because of the low PAR in the understory at my site.

The light response curves show low rates of photosynthesis in July even when leaves are exposed to high light (Fig. 17A) and this corresponds to the low *A* found in July, when ambient PAR was at its lowest level. The low rates, both in the field, and in the light response curves, suggest that AO may have down regulated photosynthesis during this time of the year. Since chlorophyll amounts remained unchanged throughout the season, but %N decreased, this down-regulation may have resulted from the inactivation of RUBISCO (Björkman 1968; Chen et al. 2014)

as well as its loss if No was exported to other plant tissues, such as roots. In October, ambient rates of photosynthesis and rates from the light response curves increased back to levels last seen in April, suggesting that once high light reaches the leaves in the fall, AO can reactivate RUBISCO and achieve higher rates of *A*. In addition the lower *A* in my study compared to Naumann et al. (2010), may have resulted from diffusional limitations due to low g_s because of the low PAR in the understory.

Daily Carbon Gain

Calculations of daily carbon gain show that AO takes advantage of its extended leaf phenology by acquiring most of its carbon in the spring before the canopy is leafed out and that it also gains significant amounts again after the canopy has lost its leaves in October. This finding is in line with the hypothesis by Brym et al. (2011) that AO takes advantage of early spring light for much of its carbon uptake, but contrasts sharply with the findings of Fridley (2012) who suggested that AO and other woody exotics gain a significant amount of their annual carbon in the autumn after canopy leaf fall.

Throughout the summer (June to September) daily carbon uptake was minimal and even negative due to the heavy shading during these months. Many herbaceous understory plants, especially those classified as summergreens, make use of the same strategy as AO (Neufeld and Young 2014). That is, they gain most of their annual carbon during the high light period in spring before the canopy leafs out, and then essentially “idle” during the summer, barely at or above the light

compensation point, gaining very little carbon if any at all (Neufeld and Young 2014). Autumn olive is likely using a similar strategy, where it builds up carbohydrate reserves in the spring when rates of photosynthesis are at their highest, and uses these photosynthates to sustain respiratory costs during shaded summer months. Then, in the autumn, when the canopy leaves fall off and light levels rise again, it makes use of a second time period to assimilate more carbon.

The two high light seasons together provide AO with most of its annual carbon. In my study, if you assume similar days to the diurnals over the spring and autumn months when the canopy is leafless or near so, a crude calculation would show that AO gains 90% of its annual carbon budget in the spring (April and May) and fall (October), and only 10% in the summer (June-September). Thus, these two high light seasons are crucial to the ability of AO to persist and thrive in an understory environment in the southern Appalachians.

Conclusion

Invasive plant species alter the composition and functioning of native ecological systems, and are estimated to cost the United States over $138 B per year to manage and control. Understanding the mechanisms which facilitate invasion and allow persistence in their new habitats is crucial for making important management and eradication decisions by land managers, and for predicting future invasions. The results of my research have shown that autumn olive is capable of persisting and rapidly spreading in varying light environments, including the shady understories of southern Appalachian forests. The two main mechanisms identified

in this research effort that allow autumn olive to dominate forest understories are (1) its extended phenology, which allows it to gain carbon in the spring and fall when the overstory canopy is leafless and light levels are high enough to support significant carbon assimilation, and (2) the ability to fix nitrogen, which may provide a competitive edge over native congeneric species that do not fix nitrogen. It is also able to re-allocate resources from sexual to asexual reproduction in the understory, further contributing to its ability to spread in low light environments. Future research should include studying the exact mechanisms by which it reproduces vegetatively and how its invasiveness might be affected by climate change.

References Cited

Ahmad SD, Sabir SM, Zubair M (2006) Ecotypes diversity in autumn olive (*Elaeagnus umbellata* Thunb): A single plant with multiple micronutrient genes. Chem Ecol 22:509-521

Björkman O (1968) Carboxydismutase activity in shade-adapted and sun-adapted species of higher plants. Physiologia Planatrum 21:1-10

Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J Ecol 83:887-889

Bonilla NO, Pringle EG (2015) Contagious seed dispersal and the spread of avian dispersed exotic plants. Biol Invasions 17:3409-3418

Bordeleau LM, Prévost D (1994) Nodulation and nitrogen fixation in extreme environments. Plant Soil 161:115-125

Boring LR, Swank WT (1984) Symbiotic nitrogen fixation in regenerating black locust (*Robinia pseudoacacia* L.) stands. For Sci 30:528-537

Brym ZT, Allen D, Ibáñez I (2014) Community control on growth and survival of an exotic shrub. Biol Invasions 16:2529-2541

Brym ZT, Lake JK, Allen D, Ostling A (2011) Plant functional traits suggest novel ecological strategy for an invasive shrub in an understory woody plant community. J Appl Ecol 48:1098-1106

Bull TA (1968) Expansion of leaf area per plant in field bean (*Vicia fabia* L.) as related to daily maximum temperature. J Appl Ecol 5:61-68

Caffarra A, Donnelly A (2001) The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. Int J Biometeorol 55(5):711-721

Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Front Ecol Environ 2:436-443

Catling PM, Oldham MJ, Sutherland DA, Brownell VR, Larson BMH (1997) The recent spread of autumn-olive, *Elaeagnus umbellata*, into Southern Ontario and its current status. Can Field Nat 111:376-380

Chazdon RL (1988) Sunflecks and their importance to forest understorey plants. Adv Ecol Res 18:1-63

Chen H, Matter SF (2017) Quantification of changes in light and temperature associated with invasive amur honeysuckle (*Lonicera maackii*). Am Midl Nat 177:143-152

Cohen E, Okon Y, Kigel J, Nur I, Heins Y (1980) Increase in dry weight and total nitrogen content in *Zea mays* and *Setaria italic*a associated with nitrogen-fixing *Azospirillum* spp. Plant Physiol 66:746-749

Craine JM, Brookshire ENJ, Cramer MD, Hasselquist NJ, Koba K, Marin-Spiotta E, Wang L (2015) Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. Plant Soil 396:1-26

Delwiche CC, Zinke PJ, Johnson CM, Virginia RA (1979) Nitrogen isotope distribution as a presumptive indicator of nitrogen fixation. Bot Gaz 140:S65-S69

Díaz-Barradas MC, Zunzunegui M, Álvarez-Cansino L, Esquivias MP, Collantes MB, Cipriotti PA (2015) Species-specific effects of the invasive *Hieracium pilosella* in Magellanic steppe grasslands are driven by nitrogen cycle changes. Plant Soil 397:175-187

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621-631

Dornbos DL Jr, Martzke MR, Gries K, Hesselink R (2016) Physiological competitiveness of autumn olive compared with native woody competitors in open field and forest understory. For Ecol Manag 372:101-108

Edgin B, John EE (2001) Control of autumn olive (*Elaeagnus umbellata* Thunb.) at Beall Woods Nature Preserve, Illinois, USA. Natural Areas Journal 21:386-388

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78:9-19

Exec. Order No. 13112, 3 C.F.R. 6183-6186 (1999)

Exec. Order No. 13751, 3 C.F.R. 6183-6186 (2016)

Farmer RE (1968) Sweetgum dormancy release – effects of chilling photoperiod and genotype. Physiologia Planatarum 21:1241-1248

Flynn DFB, Wolkovich EM (2018) Temperature and photoperiod drive spring phenology across all species in a temperate forest community. New Phytol 0:1-10

Fordham IM, Clevidence BA, Wiley ER, Zimmerman RH (2001) Fruit of autumn olive: A rich source of lycopene. Hort Sci 36:1136-1137

Fridley JD (2012) Extended leaf phenology and the autumn niche in deciduous forest invasions. Nature 485:359-362

Funk DT, Schlesinger RC, Ponder F Jr (1979) Autumn-olive as a nurse plant for black walnut. Bot Gaz 140:S110-S114

Gallagher JN (1979) Field Studies of Cereal Leaf Growth. J Exp Bot 30:625-636

Gallinat AS, Primack RB, Wagner DL (2015) Autumn, the neglected season in climate change research. Trends Ecol Evol 30:169-176

Geiger R (1950) The climate near the ground. Harvard University Press, Cambridge, Massachusetts

Ghelardini L, Santini A, Black-Samuelsson S, Myking T, Falusi M (2010) Bud dormancy relase in elm (*Ulmus* spp.) clones – a case study of photoperiod and temperature responses. Tree Physiol 30:264-274

Hardy RW, Holstein WF, Jackson EK, Burns RC (1968) The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol 43:1185-1207

Herberling JM, Jo I, Kozhevnikov A, Lee H, Fridley JD (2017) Biotic interchange in the Anthropocene: strong asymmetry in East Asian and eastern North American plant invasions. Glob Ecol and Biogeo 26:447-458

Hobbie EA, Hobbie JE (2008) Natural abundance of ^{15}N in nitrogen-limited forests and tundra can estimate nitrogen cycling through mycorrhizal fungi: a review. Ecosystems 11:815-830

Ibáñez I, Primack RB, Miller-Rushing AJ, Ellwood E, Higuchi H, Lee SD, Kobori H, Silander JA (2010) Forecasting phenology under global warming. Philos Trans R Soc B Biol Sci 365:3247-3260

Ibáñez I, Silander JA Jr, Allen JM, Treanor SA, Wilson A (2009) Identifying hotspots for plant invasions and forecasting focal points of further spread. J Appl Ecol 46: 1219-1228

Jones FR, Tisdale WB (1921) Effect of soil temperature upon the development of nodules on the roots of certain legumes. J Agric Res 22:17-37

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164-170

van Kleunen M, Weber E, Fischer M (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. Ecol Lett 13:235-24

Kobe RK (1999) Light gradient partitioning among tropical tree species through differential seedling mortality and growth. Ecology 80:187-201

Körner C, Basler D (2010) Phenology under global warming. Science 327:1461-1462

Legros L, Smith DL (1994) Root zone temperature sensitivity of nitrogen fixing and nitrate supplied soybean [*Glycine max* (L.) Merr. Cv. Maple Arrow] and lupin (*Lupinus albus* L. cv. Illtra) plants. Environ Exp Bot 34:117-127

Linkosalo T, Häkkinen R, Hänninen H (2006) Models of the spring phenology of boreal and temperate trees: is there something missing? Tree Physiol 26:1165-1172

Lynch DH, Smith DL (1994) The effects of low root zone temperature stress on two

soybean (*Glycine max*) genotypes when combines with *Bradyrhizobium* strains of varying geographic origin. Physiologia Plantarum 90:105-113

McCall LJ, Walck JL (2014) Dispersal characteristics of two native and two nonnative fleshy-fruited sympatric shrubs. Castanea 79:88-99

Monsi M, Murata Y (1970) Development of photosynthetic systems as influenced by distribution of matter. Proc IBP/PP Tech Meeting, Tfebon, Czechoslovakia, pp 115-129

Naumann JC, Bissett SN, Young DR, Edwards J, Anderson JE (2010) Diurnal patterns of photosynthesis, chlorophyll fluorescence, and PRI to evaluate water stress in the invasive species, *Elaeagnus umbellata* Thunb. Trees 24:237-245

Neufeld HS, Young DR (2014) Ecophysiology of the herbaceous layer in temperate deciduous forests. In: Gilliam FS (ed) The herbaceous layer in forests of Eastern North America, 2nd edn. Oxford University Press, New York, pp 35-95

Nobel PS, Hartsock TL (1981) Development of leaf thickness for Plectranthus parviflorus- Influence of photosynthetically active radiation. Physiol Plant 51:163-166

Orr SP, Rudgers JA, Clay K (2005) Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. Plant Ecol 181:153-165

Pacala SW, Canham CD, Saponara J, Silander JA Jr, Kobe RK, Ribbens E (1996) Forest models defined by field measurements: estimation, error analysis and dynamics. Ecol Monogr 66:1-43

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst 37:637-669

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52: 273-288

Polgar CA, Primack RB (2011) Leaf-out phenology of temperate woody plants: from trees to ecosystems. New Phytol 191:926-941

Porra RJ (2002) The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. Photosynth Res 73:149-156

Pyšek P, Jarošik V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vilà M (2012) A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. Glob Chan Biol 18:1725-1737

Rauschert E (2010) Survivorship curves. Nat Education 3:18

Reich PB, Ellsworth DS, Walters MB (1998) Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. Funct Ecol 12:948-958

Reichard SH (1997) Prevention of invasive plant introductions on national and local levels. In: Luken JA, Thieret JA (eds) Assessment and management of plant invasions. Springer, New York, pp 215-227

Reichard SH, White P (2001) Horticulutre as a pathway of invasive plant introductions in the United States. BioScience 51:103-113

Robinson D (2001) $\delta^{15}N$ as an integrator of the nitrogen cycle. TRENDS Ecol Evol 16:153-162

Roughley RJ, Date RA (1986) The effect of strain of *Rhizobium* and of temperature on nodulation and early growth of *Trifolium semipilosum*. Exp Agric 22:123-131

Schwartz MD, Reiter BE (2000) Changes in North American spring. Int J Climatol 20:929-932

Sharkey TD, Savitch LV, Butz ND (1991) Photometric method for routine determination of k_{cat} and carbamylation of rubisco. Photosynth Res 28:41-48

Shiflett SA, Zinnert JC, Young DR (2017) Functional traits of expanding, thicket forming shrubs: contrasting strategies between exotic and native species. Ecosphere 8:1-15

Spriggs AC, Stock WD, Dakora FD (2003) Influence of mycorrhizal associations on foliar $\delta^{15}N$ values of legume and non-legume shrubs and trees in the fynbos of South Africa: implications for estimating N_2 fixation using the ^{15}N natural abundance method. Plant and Soil 255:495-502

Smith LM (2013) Extended leaf phenology in deciduous forest invaders: mechanisms of impact on native communities. J Veg Sci 24:979-987

Sullivan NH, Bostad PV, Vose JM (1996) Estimates of net photosynthetic parameters for twelve species in mature forests of the southern Appalachians. Tree Physiol 16:397-406

Szafoni RE (1991) Vegetation management guideline: autumn olive, *Elaeagnus umbellata* Thunb. Nat Areas J 11:121-122

Traveset A, Richardson DM (2006) Biological invasions as disruptors of plant reproductive mutualisms. Trends Ecol Evol 21: 208-216

Unkovich M (2013) Isotope discrimination provides new insight into biological nitrogen fixation. New Phytol 198:643-646

Warren RJ II, Candeias M, Labatore A, Olejniczak M, Yang L (2018) Multiple mechanisms in woodland plant species invasion. J Plant Ecol rty010:1-9

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002) Plant Ecological Strategies: Some leading dimensions of variation between species. Annu Rev Ecol Syst 33:125-159

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niiemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. (2004) The worldwide leaf economics spectrum. Nature 428:821-827

Yates ED, Levia DF Jr, Williams CL (2004) Recruitment of three non-native invasive plants into a fragmented forest in southern Illinois. For Ecol Manag 190:119-130

Zhang F, Dashti N, Hynes RK, Smith DL (1996) Plant growth promoting rhizobacteria and soybean [*Glycine max* (L.) Merr.] nodulation and nitrogen fixation at suboptimal root zone temperatures. Ann Bot 77:453-459

Zhang X, Tarpley D, Sullivan JT (2007) Diverse responses of phenology to a warming climate. Geophys Res Lett 34:1-5

Zinnert JC, Shiflett SA, Vick JK, Young DR (2013) Plant functional traits of a shrub invader relative to sympatric native shrubs. Ecosphere 4:1-11

Vita

Emily Caroline Riffe was born in Charlotte, North Carolina in 1995. In 2013 she graduated from Cabarrus-Kannapolis Early College High School and Rowan Cabarrus Community College with her Associate Degree in Arts. She attended North Carolina State University where she graduated in 2016 with a major in Plant Biology and a minor in Horticulture Science. During her time there, she worked in multiple plant science laboratories with professors Dr. Deyu Xie and Dr. Hamid Ashrafi. She also completed an internship at the Plants for Human Health Institute in Kannapolis, NC during the summer of 2015 where she worked with Dr. Jessica Schlueter. After her graduation she began work with Dr. Howard Neufeld at Appalachian State University on plant ecophysiology and completed her Master's in August of 2018. Upon completion of her degree, she accepted a position as a research technician at the Washington State University Tree Fruit Research and Extension Center located in Wenatchee, WA.